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A STUDY OF THE EFFECTS OF TOTAL AND PARTIAL BODY RADIATION ON IRON METABOLISM AND HEMATOPOIESIS

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ABSTRACT

This report covers further observations on patients who have received total body radiation in the course of treatment of advanced cancer. The data which was previously submitted in table form has been re-analyzed and presented in graphic form for simplicity.

In the course of study of the significance of radioiron tracer studies as an index of radiation effect, over 700 such studies on more than 300 patients have been carried out. The interpretation of the data of patients with a variety of hematopoietic disorders is presented as back ground for evaluating radioiron tracer studies in radiation effects.

The role of radioiron tracer studies as a measure of radiation damage has been evaluated in cancer patients receiving total body radiation and limited-field radiation.

Extension of radioiron tracer studies to provide a test for residual radiation damage is described and discussed, even though a preliminary experiment failed to indicate a useful technique.

Procedures have been established for the identification and quantitative estimation of enzyme systems in circulating red blood cells with a view to establishing a biologic dosimeter.

Continuing experience in the use of total body radiation for the treatment for some forms of advanced cancer has resulted in an increasing volume of data and some revision of expression of observations without altering the tentative conclusions that have been previously drawn. These have had to do with tolerance values for whole body radiation delivered under clinical therapeutic circumstances.

There is limitation on the importance of these values in that extrapolation to radiation exposures under emergency or catastrophic conditions can be no better than the measurement or estimation of quantity of radiation under such conditions. Even if comprehensive physical measurements of radiation were obtained, the circumstances of time, volume and geometry which govern the biologic aspects of dose may greatly alter the significance of an exposure to any given number of roentgens.

For this reason particular attention has been given to biologic responses which might reflect the quantitative physical exposure.

Several studies have been completed since the progress report for the period ending 21 August 1955 (AFSWP-810). From the beginning it was evident that radioiron tracer studies as a clinical laboratory procedure would have to be evaluated on a broad basis if the data related to radiation effects were to be meaningful. More than 700 tests have been done and the data studied. Appendix 1 is a compilation of this material in a manuscript that was solicited for the Brazilian journal

Resenha. It may be noted that the test has established its value in the dynamic evaluation of erythropoiesis in all conditions studied.

A development of the studies on iron metabolism is contained in Appendix 2. This is a paper by a graduate student, R. Tinguely, working in the department. It presents a simple procedure for determining the residual iron-binding capacity of serum. This has been in routine use for the past few months, primarily to determine how frequently the turnover rate of iron is limited by the capacity of the serum iron transport mechanism. To date, no sera have been analyzed which did not have ample residual binding capacity.

The use of iron tracer studies for the evaluation of radiation damage in clinical medicine has been prepared for presentation to the American Radium Society in April 1955. A previous report on the therapeutic aspects of total body radiation was made to this Society two years ago and is due to appear in the March issue of the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine. This manuscript is included as Appendix 3. This report summarizes the experience of the department with reference to the effects of whole and partial body radiation on the iron turnover test results. It is concluded that the test provides a rapid and useful index of body tolerance to systemic depressant agents, but is of little or no value in the assessment of systemic tolerance to protracted limited-field radiotherapy.

Appendix 3 also contains a detailed account of an attempt to unmask residual radiation damage following apparent recovery of rabbits from a total body radiation dose of 500r. The proposal here was that the normal hematopoietic response to anoxia might be impaired following radiation. If this were so, the damage might be detected by radioiron tracer studies in animals previously radiated and subjected to anoxia at the time of test. The results have been inconclusive. Decreased functional reserve as a residual effect of radiation has not been demonstrated but it is considered possible that an effect might be encountered if the interval between radiation and stressing by anoxia is increased.

As one effort to establish a "biological dosimeter", Dr. D. A. Rappoport has suggested that the enzyme systems of the circulating red blood cells might show a quantitative relationship to exposure of total body x-radiation.

Two premises were made as the basis of erythrocyte enzymic dosimetry.

1. The long life-span of red cells will permit examination of cells which have been exposed to x-rays at prolonged periods after radiation (2 to 4 weeks).

2. The inability of erythrocytes to resynthesize new proteins (or enzymes) indicated that damaged enzymic systems (due to x-rays) will not be repaired; hence they will reflect x-ray damage for long intervals after exposure.

Appendix 4 is a summary report describing the work in progress directed toward the ultimate objective of finding a biological or enzymic dosimeter.

A radiation building has been completed and a 2 MeV Van de Graaff x-ray generator installed. Preliminary calibration and dosimetry procedures have been carried out and clinical usage including total body radiation has begun. The treatment room measures 35' x 18' and is designed to permit treatment by stationary or rotation technics at distances up to 8 meters.

1. Observations on patients receiving total body radiation will be continued, giving particular attention to protraction over very short to very long intervals with basic observations on clinical systemic response and the effects on peripheral blood and iron metabolism.

2. Material will be collected for the study of tetanus antibody response in patients receiving whole body radiation.

3. The study of enzyme systems of red blood cells as possible biologic dosimeters will be extended.

4. The possibilities for using stress technics to reveal masked metabolic defects following apparent recovery from radiation will be investigated further.

5. The aim of studies in the field of radiation effects is to gauge the hazard of exposures that may be encountered under any circumstance. Despite the volume of data that has been amassed by the very numerous observers, the quantitative and qualitative aspects of response of living tissue can be measured or described in only the broadest terms. The failure to establish clear and dependable relationships with precise physical data may be due to the inadequacy of our observations of biologic effects, or to studying inappropriate biologic systems. Such uncertainty is not peculiar to radiobiology.

A similar problem exists in gerontologic studies. There is no system of definition or measurement for senescence or aging but these processes are associated with an increased vulnerability to hazard and liability to death. Some similarity to the delayed effects of radiation is evident.

Consideration will be given to the adaption of technics of studies of the aging process to the problem of identifying radiation effects.

APPENDIX #1

Methods and Applications of Radioiron Studies

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Radioactive iron has been used for about fifteen years to investigate iron metabolism (1-5). The intravenous iron turnover test as a specific diagnostic aid for the study of hemoglobin production was described by Huff et al in 1950 (6). That group and others have studied many clinical and experimental phases of iron metabolism with this standardized technique, and utilizing other equipment and techniques (7-32). A slight modification (33) has made it possible to perform the test more simply on a routine laboratory basis. Using the technique as described below, over 700 tests on more than 300 patients have been performed in this department, permitting some generalizations to be drawn.

The radioiron turnover test in its simplest form provides a method for determining the daily production of hemoglobin, and for estimating the functional status of the erythropoietic system. It is a useful aid in the differential diagnosis of anemia, and also provides a quantitative statement of the severity of the disorder. For example, a hemoglobin level of 8 grams per 100 ml. blood tells only that the over-all balance between hemoglobin production and destruction is such that the body can maintain approximately half of the normal hemoglobin concentration. Radioiron studies will indicate whether this balance results from half of the normal production, with a normal life span; or normal production with half the normal span; or perhaps twice the normal production as a response to the anemia, with a life span of the circulating erythrocytes of only one-fourth of normal.

The first part of this test, the determination of the rate at which plasma-bound iron is cleared from the circulation, can be performed in a matter of two to three hours. While this part of the test is rarely sufficient in itself to complete the quantitative diagnosis, it can serve as a very rapid index of change of hemoglobin production. When hemoglobin production is depressed by large doses of total body radiation or cancer chemotherapeutic agents, the change can be readily documented by successive iron turnovers within twenty-four hours. Detailed analysis of the uses and advantages of radioiron tracer studies in clinical practice will be given below in the section on "Interpretation".

Part I

IRON METABOLISM

The Basis for the Use of the Tracer

The radioiron turnover test and its modifications can be logically derived and interpreted from a consideration of normal iron metabolism (34-36). This is shown schematically in Figure 1.

Normal metabolism is a "closed system", i.e., the body tends to maintain its iron supply with a minimum of excretion, and absorbs just enough to compensate for the small losses. A normal 70 Kg. man has about 3.5 - 4.5 grams of iron, about 2.5 grams as hemoglobin iron, .002 - .003 grams as circulating plasma iron, and the remainder as storage iron or in a relatively fixed status in myoglobin and the cytochrome respiratory enzymes. The hemoglobin cycle (synthesis of new hemoglobin and breakdown of old red cells) is responsible for the major turnover of iron. Since 2.5 grams of iron are present at all times in red cells, and since these cells live approximately 120 days, then about 0.83%, or about 21 mg., of this iron must be released from destroyed cells and a comparable amount re-utilized in new hemoglobin formation each day. Iron is transported from the site of destruction to the erythropoietic tissue by the plasma. With the plasma containing 2-3 mg. of iron at any given time, yet transporting at least 21 mg. per day, there must be a replacement at least 7-10 times per day. In spite of its small iron content, the plasma transport system is of crucial importance. It is also readily available for sampling and serial analysis. The iron turnover test is designed to measure the rate of the plasma iron replacement, and the rate and extent of the utilization of this iron for hemoglobin formation.

Between 90 and 100% of the total iron in the plasma occurs as transport iron. This portion is bound reversibly to a beta globulin, the IV-7 fraction of Cohn's classification, also named transferrin. A portion of this protein-bound iron is removed by the bone marrow and storage depots with each circulation of the blood.

Meanwhile, the plasma iron concentration is maintained by iron from the breakdown of old red cells, to a lesser extent from the storage depots, and by a still smaller amount from intestinal absorption. This concentration is not constant, being subject to diurnal variations as well as reflecting disease states (37-39).

The iron removed from the plasma by erythropoietic tissue is rapidly incorporated into hemoglobin and released back to the circulation in the red blood cells. Usually 80 - 95% of the iron cleared from the plasma is so utilized, the other 5 - 20% presumably having gone to the storage depots or to fixed systems. (Fig Some of this radioiron reappears in the circulation in hemoglobin in as short a time as 3-12 hours while maximum reappearance occurs within 5-10 days. There is good evidence that mature red cells cannot exchange hemoglobin iron with plasma iron, although reticulocytes apparently can incorporate some iron directly from the plasma. It is possible that the rapid clearance of plasma iron noted in severe iron deficiency or hemolytic anemia results in part from direct incorporation into reticulocytes.

A variable amount of iron exists in the reticulo-endothelial storage depots, primarily of the liver, spleen, and bone marrow. In a normal state, the storage depots function effectively to maintain the concentration of transport iron, accepting iron when the plasma concentration is high and yielding iron when the plasma concentration falls. The turnover of the plasma iron is therefore due in part to this equilibrium with storage iron, although normally this is of much less magnitude than that due to the hemoglobin cycle.

A small amount of iron occurs in apparently fixed forms in myoglobin, the cytochrome enzymes, and to a lesser extent in other cellular components. Present evidence indicates that this iron is probably not in equilibrium with the remainder of the body iron, but is released only with cell destruction or exfoliation. Loss of body iron normally occurs largely by this method, urinary, fecal, and sweat losses being small though measurable and of physiologic importance (21, 40-43). Women have a regular loss of hemoglobin iron during menses.

The rate of replacement of iron is controlled in the intestinal mucosa by an iron-binding enzyme, apo-ferritin. Since there is no physiological mechanism for excreting excess iron, the body content must be limited by absorbing only enough from ingested food to replace actual loss. Apo-ferritin usually has attached a saturating quantity of iron, this apo-ferritin iron complex being called ferritin. When the body stores of iron are decreased, ferritin releases some of its iron to the plasma, and can then absorb an equal quantity from the intestinal contents. There is uncertainty whether the release of iron by ferritin to the plasma is controlled directly by the plasma iron concentration, or indirectly by the status of the storage depots (44). Hemochromatosis is apparently a disease associated with uninhibited absorption of iron (20, 27, 32, 45-47).

PROCEDURE

A purpose of the iron turnover test is to determine the amount of hemoglobin formed per day. To do so, it is necessary to know: (a) the plasma iron concentration, (b) the rate at which this iron leaves the plasma, (c) the percentage of this iron which is incorporated into hemoglobin, and (d) the blood volume. The iron concentration is determined by chemical procedures (48, 49). The remaining three items are determined using radioactive tracer techniques.

If a functionally negligible quantity of a substance is distributed uniformly throughout a metabolic pool of the same substance, it will react exactly as does the native material, and will not alter the normal reactions. If the added material is radioactive, its subsequent distribution may be determined by appropriate detection techniques. Meticulous attention must be paid to consideration that a true tracer study is being performed: (a) that the tracer is truly identical to the substance being traced; (b) that the tracer is not in such large quantity as to measurably increase the material present, which may alter its subsequent distribution; (c) that the sampling procedure does not change the system to any measurable extent; and (d) that the radiation does not affect the system.

[illegible] radioisotope attached to the

IV-7 plasma fraction. This iron protein complex can be injected intravenously to become distributed uniformly in the existing plasma iron. Samples of plasma can then be taken at intervals to determine the disappearance rate, and later whole blood samples to evaluate reappearance. Initially the tracer was prepared by incubating the patient's plasma with buffered iron salts at room temperature for at least ten minutes, allowing the iron to become attached to transferrin, before reinjection (6). It has been shown (33) that iron transfers from iron citrate at pH 7 to the iron binding protein sufficiently rapidly as to permit the direct injection of radioiron citrate. Latent iron binding capacity sufficient to accept the minute quantity of iron injected is present even in conditions associated with near saturation of the IV-7 fraction (50-51). This technique has the advantage that the tracer may be prepared in bulk and stored in withdrawal bottles for several weeks. The possibilities of bacterial contamination and of denaturation of protein during the preparation procedures are also reduced. Iron citrate can be prepared (33) from iron chloride as received from the Atomic Energy Commission, containing about one microgram of iron per microcurie of radioactivity. A tracer dose of four microcuries will contain a quantity of iron small (4-8 micrograms) in comparison to normal plasma iron levels (about 100 micrograms per 100 ml. of plasma). The radiation received by the body in the course of an iron turnover is a small fraction of that received during a routine chest roentgenogram. The taking of five or ten blood samples of two-three ml. each is not likely to reduce the blood volume enough to measurably affect hemoglobin production. Thus the criteria for a tracer study are well met.

The radioactive tracer, consisting of protein-bound radioiron or radioiron citrate, is introduced into the general circulation, where it usually equilibrates within 5-10 minutes. With each circulation of the blood, a portion of the radioactive as well as of the normal iron will be removed. While non-radioactive iron enters the circulation to maintain the plasma concentration, the radioactive material is constantly being depleted, a similar percentage of the residual being

removed with each circulation. Since a portion only of the residual is removed, all radioactive molecules can, in theory at least, never be removed. This is similar to cutting a string in half, then cutting one of the pieces in half again, etc. In theory there will always be a piece left to cut in half, although in practice this will soon become microscopically small. Since it is impossible to state a time when all the iron has disappeared, two conventional expressions of rate are used. One is to state the percentage removed in a convenient time period, e.g., 40% per hour. The other is to give the time required to reach a given percent of the initial activity, e.g., one-half. This later method, expressed as half-time, is most frequently used in medical literature and will be used here.

After the injection of radioiron, samples of plasma are obtained at intervals, and their radioactivity determined. The simplest way to obtain the clearance half-time is to plot the activity of the plasma samples against time on semi-logarithmic graph paper (Figure II). If the activity is decreasing exactly by halves, a straight line will be obtained, from which the half-time is determinable.

The disappearance curve may not be a perfect straight line on semi-logarithmic graph paper, since some radioiron, which initially is removed by the storage depots, will be released back into the plasma. The activity may decrease less rapidly than expected, giving a curved line with a steadily more gentle drop. In our series we have encountered very few disappearance plots which are not almost straight lines, although some workers report quite consistent curvature. If iron citrate solution is kept for many months, it forms a colloid containing iron hydroxide. Such suspensions, and also solutions of iron chloride, will consistently yield curved disappearance plots which are of no value for these tracer studies.

To determine the daily clearance of iron from the entire plasma pool, it is necessary to know the size of the plasma pool. This is obtained incidentally but with considerable reproducibility from the isotopic disappearance rate test. Since

a known quantity of radioactivity is added to the plasma, a subsequent determination of the activity of a plasma sample permits a determination of the degree of dilution of the injected material. The fewer the plasma counts, the greater has been the dilution, and therefore the greater the plasma volume. The most reliable value is obtained by drawing the disappearance curve backward to estimate the radioactivity per milliliter of plasma just after injection and using this value to obtain a dilution factor.

It is also necessary to count at least one sample of whole blood taken early during the disappearance test (Figure II). This will permit a calculation of the radioactivity per milliliter of whole blood at zero time, which will be used later to determine percentage utilization in hemoglobin production. Simultaneously, a comparison of the radioactivity per milliliter of whole blood, and per milliliter of plasma, gives an estimate of the peripheral hematocrit.

To obtain percentage utilization, whole blood samples are taken several days after the disappearance test. By this time all of the radioiron has been removed from the plasma. Some has been incorporated into hemoglobin and released back into the circulation in red blood cells. By dividing the whole blood activity of any sample several days after the disappearance test, by the activity of the zero time whole blood, the percentage incorporation can be computed. It has been shown that the ratio of plasma volume to red cell mass in the whole body is not identical to that of the peripheral venous blood (52, 53). The ratio of body hematocrit to peripheral hematocrit is quite constant at about 0.91. The red cell mass is thus about 86% of that calculated from the plasma volume and the peripheral hematocrit. As a result, complete utilization of radioiron for hemoglobin production would give a calculated utilization of 116%. The value obtained, even if over 100%, is still the correct figure to use for subsequent calculations, since it indicates that a known plasma pool is contributing iron to a red cell mass smaller than calculated.

The data obtained experimentally are:

1. Plasma iron concentration - Fe_{pc} (ug. %)
2. Plasma iron disappearance half-time - T/2 (hours)
3. Plasma volume - P.V. (ml.)
4. Hematocrit, either Wintrobe or isotope - Hct. (%)
5. Hemoglobin, routine lab procedure - gm. %.
6. Percent maximum incorporation of radioiron in circulating erythrocytes.

Calculated data are:

1. Daily Iron Clearance. Knowing the plasma iron concentration and the rate of removal (and replacement), the amount of iron removed per day from the plasma can be computed with the use of calculus. The formula obtained is:

Daily iron clearance per 100 ml. of plasma (in ug.) =

$$\frac{0.693 \times 24 \text{ hours} \times Fe_{pc} \text{ (in ug. \% plasma)}}{T/2 \text{ (hours)}} = \frac{16.6 \times Fe_{pc}}{T/2}$$

Daily clearance per 100 ml. of whole blood (in ug.) =

$$\left(\frac{16.6 \times Fe_{pc}}{T/2}\right) \quad (1 - Hct)$$

These calculations are subject to errors resulting from diurnal variations in plasma iron concentration and in clearance rate (38).

2. Daily Hemoglobin formation. The iron cleared from the plasma per day, times the percent incorporated into red cells, gives the quantity of iron utilized per day for hemoglobin formation. Since there are 3.34 mg. of iron in 1 gram of hemoglobin, the daily hemoglobin production is:

Grams Hgb formed per 100 ml. whole blood =

Daily iron clearance per 100 ml. whole blood (mg/day) x $\frac{\text{percent utilization}}{3.34}$

3. Percentage daily replacement of hemoglobin. The amount replaced daily divided by the total amount present:

$$\% \text{ Replacement/day} = \frac{100 \times \text{grams replaced}}{\text{grams present}}$$

4. Average life span is inversely related to the percentage daily replacement:

$$\text{Average life span (days)} = \frac{100}{\% \text{ replacement/day}}$$

5. Total hemoglobin production for the body can be approximately calculated.

Blood volume (B.V.) is approximately equal to $\frac{\text{P.V.}}{\text{Peripheral Plasmacrit}}$

Grams Hgb. formed/day - B.V. (in 100 mls) x Grams Hgb. formed per 100 ml. whole blood.

6. A composite formula for calculating red cell life span directly is:

$$\text{Red cell life span (days)} = \frac{\text{Hgb. x T/2 x 175}}{\text{Fe}_{pc} \times \% \text{ uptake} \times (1\text{-Hct})}$$

TECHNIQUE

1. Withdraw 3-5 cc. of blood into a heparinized syringe for whole blood and plasma samples (1.0 - 2.0 cc.) for the determination of natural radioactivity.

2. Inject intravenously 5.0 - 10.0 cc. of Fe^{59} citrate solution containing 4 microcuries of Fe^{59}. Avoid venostasis.

3. Obtain heparinized blood samples (3 - 5 cc.) at 5, 15, 30, 60, and 120 minutes after injection. Avoid venostasis. Pipette 1.0 or 2.0 cc. aliquot of whole blood from the 15 minute sample, and 1.0 or 2.0 cc. of plasma from all samples into counting tubes.

4. Count all samples for steps 1 and 3. Subtract background counts.

5. Plot plasma activity (log scale) against time (linear scale) on semi-log paper.

6. Extrapolate to obtain zero time plasma activity. Determine disappearance half-time ($T\frac{1}{2}$) of plasma Fe^{59}.

7. Calculate plasma volume: $\text{P.V.} = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. plasma}}$

8. Calculate hematocrit from activity of 15 minute samples:

$$\text{Hct} = 1 - \text{Plasmacrit} = 1 - \frac{\text{Counts/cc. whole blood}}{\text{Counts/cc. Plasma}}$$

9. Calculate zero time whole blood activity by proportionality:

Zero time counts/cc. Whole Blood = Zero time counts/cc. Plasma x Plasmacrit

10. Calculate blood volume:

$$\text{B.V.} = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. whole blood}}$$

11. Pool plasma samples, determine iron concentration on duplicate aliquots.

12. Calculate daily plasma iron clearance.

$$\text{Fe cleared/day} = \frac{16.6 \times \text{Fe}_{\text{PC}}\ \text{mg\%} \times \text{P.V. (in 100 ml's.)}}{\text{T/2 (hours)}}$$

13. Obtain blood samples (2 ml., heparinized) at 3, 7, and 14 days. Count 1.0 or 2.0 ml. aliquot and hematocrit.

14. Determine approximate percent red cell incorporation of Fe^{59}:

$$\text{\% uptake} = \frac{\text{Count/ml. follow-up blood sample x 100}}{\text{Zero time count/ml. whole blood}}$$

15. Calculate Hgb. production per day:

$$\text{Gms Hgb. Produced/Day} = \frac{\text{mg. plasma iron cleared/day x \% uptake}}{3.34}$$

16. Calculate total body hemoglobin:

Blood volume (in 100 ml's) x Hgb (gm. %) = Total gms. hgb.

17. Calculate % daily replacement Hgb.:

$$\text{\% Daily Hgb. replacement} = \frac{\text{Gm. Daily Hemoglobin Production x 100}}{\text{Total Body Hgb.}}$$

18. Calculate average red cell life span:

$$\text{Average red cell life span (days)} = \frac{100}{\text{Percent daily Hgb. replacement}}$$

In the above calculations, radioactive decay has not been mentioned. All counting rates are assumed to have been corrected for background counts and radioactive decay before being used for calculations. If the counter used is very stable, decay corrections calculated on the basis of the half life of Fe^{59} of 47 days may be used. In general, it is more reliable and just as simple to keep a sample of the initial iron preparation as a standard, and to correct observed counting rates by a factor:

$$\frac{\text{Initial counting rate of standard}}{\text{Counting rate of standard on day of sample counting}}$$

For the normal adult with a normal hemoglobin level, values for the above observed and calculated data are:

Plasma iron half-time of disappearance	1 - 2 hrs.
Plasma iron concentration	60 - 110 ug. %
Hemoglobin concentration	13 - 16 gm. %
Blood volume	80 - 90 ml. Kg.
Hematocrit	40 - 50 %

Plasma volume	40-55 ml/Kg.
Percent incorporation of radioiron into hemoglobin	80-95% (corrected) in 7-10 days
Daily iron clearance/100 ml. plasma	0.7 - 0.8 mg.
Daily iron clearance/100 ml. whole blood	0.4 mg.
Daily hemoglobin formed/100 ml. whole blood	.11 - .13 gm.
Percent daily hemoglobin replacement	0.8 - 0.85%
Red cell life span	110 - 125 days

Part II

INTERPRETATION

For the purpose of interpreting the radioiron turnover data, hematopoietic disorders may be classified on a functional or dynamic basis as follows:

1. Iron deficiency anemia
2. Hemolytic anemia
3. Hypoplastic anemia
4. Blood loss anemia
5. Polycythemia
6. Miscellaneous conditions
   a. Pernicious anemia
   b. Mediterranean anemia
   c. Anemia associated with cancer and infections
   d. Hemochromatosis
   e. Combinations of conditions

1. Iron deficiency. The hematopoietic tissue is functionally normal and very active; the red cell life span is normal; and the plasma iron concentration is low. Because of the decreased hemoglobin concentration, the avidity of the bone marrow for iron is increased, resulting in an increased rate of plasma iron clearance. With the low plasma iron concentration, the percentage removal of iron by the marrow rises still further in an attempt to increase the total iron cleared. In severe iron deficiency, the half-time may be as short as 15 minutes. This probably represents a minimum value, a limit imposed by the time required for passage of the plasma pool through the bone marrow. The rapid clearance rate in turn maintains a low plasma iron concentration, which may approach the zero level. Since the hematopoietic tissue can function normally, and is usually hyperplastic, the cleared iron is rapidly incorporated into red cells which are released into the circulation. Essentially 100% reappearance in three days has been observed. The red cell life span calculated as described above, has little meaning unless the deficiency is in a steady state. During treatment, many times the normal

amount of hemoglobin may be produced per day.

2. Hemolytic anemias. Iron supplies are adequate, and hemoglobin production is normal or increased. The red blood cells may be inherently defective or cells may be normal with excess destruction caused by extraneous agents. In response to the resulting anemia, the clearance of iron by the marrow is increased, giving a half-time which may be as short as 20 minutes. The rapid clearance tends to reduce the plasma iron concentration, while the adequate iron stores and return of iron from increased red cell destruction tend to maintain a normal plasma iron concentration. The end result is usually a somewhat reduced concentration of serum iron (30-60 ug. %), although during hemolytic crises the concentration may rise above normal. Since the marrow can respond normally to anemia, at least from a quantitative viewpoint, it will become hyperplastic. The cleared iron will be rapidly and completely incorporated into hemoglobin, giving close to 100% reappearance in 3-4 days. During periods of relative status quo, the calculated life spans will give a close index of the degree of hemolysis, though the relationship will not hold during crises.

3. Hypoplastic anemias. Supplies of iron and other precursors for red cell production are adequate, and formed cells are adequate, but bone marrow activity is decreased. Since the utilization of iron is decreased, the plasma clearance rate is slowed, often to half-times of 4-6 hours. As a result of the decreased clearance, the plasma iron concentration rises. In such conditions, the portion of iron going to the storage depots may reach 80-95% of the iron leaving the plasma while only 5-20 percent will be used in hemoglobin formation. The methods outlined here for computing red cell life span are applicable in hypoplastic anemias except for those of acute origin when the circulating hemoglobin level has not yet come into equilibrium with the rate of production.

It is to be accented that the rate of exchange of plasma iron with storage depot iron offers an upper limit to the length of the half-time. Even with completely aplastic erythropoietic tissue, this exchange will usually limit the

half-time to 8-10 hours. The observed half-time will be a result of the two half-times--that to the storage depots and that to the marrow--according to the equation:

$$\text{Observed } T/2 = \frac{\text{Marrow } T/2 \times \text{Depot } T/2}{\text{Marrow } T/2 + \text{Depot } T/2}$$

Thus, if the marrow T/2 is 16 hours, and the depot T/2 is 8 hours, the observed plasma clearance T/2 will be:

$$\text{Observed } T/2 = \frac{16 \text{ hours} \times 8 \text{ hours}}{16 \text{ hours} + 8 \text{ hours}} = 5\ 1/3 \text{ hours.}$$

In this case the depot clearance is of considerable importance. In a normal person with a marrow clearance T/2 of 1 hour, the effect of a depot clearance T/2 of 8 hours will be small, as:

$$\text{Observed } T/2 = \frac{1 \text{ hour} \times 8 \text{ hours}}{1 \text{ hour} + 8 \text{ hours}} = 53 \text{ minutes.}$$

If the depot clearance T/2 is greater than 8 hours, the influence on the over-all T/2 will be even less.

Chronic Blood loss anemia: If the bleeding is not excessive and iron intake is adequate, blood loss anemia will present an uncomplicated pattern. In general, however, if chronic blood loss is of sufficient magnitude to cause anemia, iron deficiency will usually have been produced by the time the patient is first seen. When iron deficiency is not a factor, the supplies of all red cell precursors will be adequate, marrow function will be increased maximally in response to the anemia, but the functional red cell life span will be reduced as a result of the actual loss of cells. For the first three days of the test the data will thus be similar to that encountered in hemolytic disease; that is, very rapid clearance, somewhat reduced iron concentration, and close to complete reappearance of the cleared iron in red cells within 3-4 days. From here the patterns will vary. In hemolytic anemia the cells, even though of shortened life span, will remain in the circulation maintaining a constant level of circulating radioactivity. Even with cell destruction, the iron will be promptly reutilized, so that the blood radioactivity will be reasonably constant over many red cell life spans. Decrease in activity (other than

that due to radioactive decay) will take place slowly due to partial storage and excretion losses, with each reutilization. With blood loss however, the whole blood radioactivity will start to drop immediately because of the loss from the body of red cells of all ages. When uncomplicated, this loss will follow an exponential curve, a given percentage of all red cells being lost per day, irrespective of cell age. The rate of loss can be estimated initially from the effective red cell life span calculated from data obtained during the first three days, then subsequently confirmed from the observed rate of loss of whole blood radioactivity.

When iron deficiency has become superimposed, the pattern will be essentially the same except that the anemia is likely to be more severe, plasma iron concentration will approach zero and the T/2 will approach 15 minutes. The proof of blood loss will still rest upon the whole blood radioactivity curve over a period of weeks.

Polycythemia: This condition, whether primary or secondary, represents overproduction of red cells in the face of already excessive hemoglobin. Iron stores are adequate and red cell life span may be somewhat shortened. Because of the overactivity of the hematopoietic system, the rate of plasma iron clearance is increased, T/2's of 20-30 minutes being encountered frequently. Because of the rapid clearance rate, plasma iron concentration tends to be slightly low although normal or elevated concentrations have been observed. The red cell uptake of the cleared iron reaches approximately 100% in 3-5 days.

Pernicious anemia and Mediterranean anemia: An unusual and, at this time, unexplained combination of iron turnover constants is noted in untreated pernicious anemia and in Mediterranean anemia. This combination---rapid plasma clearance, high plasma iron concentration, and poor red cell uptake over a prolonged period of weeks---has been observed in all of nine patients with untreated pernicious anemia and five with thalassemia. With one exception (a patient with Gaucher's disease), this pattern has not been observed in any other condition. The failure

of the large quantity of cleared plasma iron to reappear in circulating hemoglobin indicates that it is not incorporated into useful erythrocytes. Three explanations have been offered to account for this discrepancy.

1. The plasma iron is removed by the immature red cell precursors, but because of "maturation arrest" is not released in circulating red cells. If this were the case, the iron would eventually have to be released back to the plasma, since the physical capacity of the cells in the marrow would soon be reached.

2. The cleared iron is initially released in erythrocytes, but most of these are of defective structure and are rapidly destroyed. The radioiron in circulating erythrocytes at any one time would be but a small part of that constantly being reutilized for new hemoglobin production. The gradually increasing blood radioactivity over a period of weeks is accounted for by the random incorporation of radioiron into the small percentage of adequate cells, where it is contained for more normal periods of time. The main objection to this explanation is that it requires the postulation of a tremendous degree of hemolysis, which is not confirmed by the quantities of excreted breakdown products of hemoglobin. The excretion of porphyrins is somewhat increased over normal, but not to the extent which would be required to support this theory.

3. The rapid clearance of plasma iron along with the elevated plasma iron concentration, represents the rapid exchange of large quantities of iron between plasma and storage depots to the exclusion of the marrow. The steady rise in red cell radioactivity is explained by the repeated opportunities of any one iron atom to be removed by the marrow, because of the rapid and continuous equilibrium of the storage depot iron with the plasma. Some evidence to indicate that this explanation may be correct, is offered from four patients with pernicious anemia. On the basis of this explanation, and of the observed plasma iron concentrations, clearance rates, and red cell incorporation curves, the quantity of storage iron was estimated. Complete utilization of this iron would have raised the circulating hemoglobin levels of these four patients to respectively 11, 13, 18, and 21 gm.%.

Following vitamin B_{12} therapy, the first two did become iron deficient at approximately the predicted hemoglobin levels, while the other two were not iron deficient when the hemoglobin concentrations stabilized at about 15-16 gm %. The labile iron storage explanation gives no indication of the origin of or mechanism for such rapid exchange, and also leaves other points unexplained, but on the basis of the available evidence is favored by the author at this time.

Anemias associated with cancer, infections, arthritis: In almost all of the anemias in this group that we have studied, a hemolytic pattern has been observed, with calculated red cell life spans approximately half of normal. Anemia associated with generalized neoplastic disease is often ascribed to decreased red cell production because of physical replacement of marrow elements by tumor. A hypoplastic pattern was observed in only three of over 200 anemic cancer patients in our experience. Probably, enlargement of the erythropoietic tissues to include peripheral marrow spaces, and extramedullary hematopoiesis, are able to compensate for a relatively slow and steady loss of normal marrow space. The explanation for the hemolytic pattern associated with these disease conditions is not indicated by radioiron studies.

Hemochromatosis: This disease apparently is caused by a defect in the mechanism controlling the absorption of iron from the intestine, and is not associated with any particular hematopoietic disorder. Iron is constantly absorbed, uninhibited by the high plasma iron concentration and excessive storage iron. Since the body has no mechanism for eliminating excess iron, the absorbed iron in turn must be stored. The plasma iron concentration is high, the clearance rate is normal, and percent uptake is reduced. Total hemoglobin production and red cell life span are normal (30).

Combined conditions in the production of anemia: Not infrequently, several deficiencies or defects will simultaneously contribute to an observed anemia. The interpretation of the iron turnover data will often be semi-empirical in such cases. For example, the normal half-time is stated to be 1-2 hours. With an

impending iron deficient state, even though the hemoglobin concentration, marrow function, and red cell life span are all normal, the plasma iron concentration will be low, and clearance rapid. Similarly, with anemia caused by hemolysis or bleeding, it is to be expected that the T/2 will be short if the marrow is capable of responding adequately to the stimulus provided by the low hemoglobin. The half-time will also be dependent upon the status of the iron stores and the duration of the anemia as well as upon its severity. With a hemoglobin of 6-7 gm. % and normal or low plasma iron concentration, a T/2 of 1½ hours would not be normal, even though this lies within the stated normal range. This would be interpreted as a hypoplastic response to an anemia of other primary etiology.

In our series, patients with the following combinations have been observed:

One with Mediterranean anemia and iron deficiency.

One with hypothyroidism, hypoplastic anemia, and iron deficiency.

Three with hemolytic anemia and hypoplasia.

Two with hemolytic anemia (one sickle cell) and iron deficiency.

Five with neoplastic disease with hemolytic anemia and iron deficiency.

One with anemia of pregnancy (megaloblastic), hemolytic disease, and excessive plasma iron concentration following large oral intake of ferrous gluconate.

One with polycythemia and iron deficiency.

In Table I are presented the types of anemia which we have studied, along with the radioiron turnover data observed.

Part III

Clinical Application of Radioiron Tracer Studies

1. Diagnostic:--Many hematopoietic disorders will be diagnosed as to type and etiology quite readily from the history and physical examination of the patient, the Wintrobe constants, and the stained blood smear. The overall severity of the disease will be evident from the hemoglobin level. Radioiron studies would then be primarily of academic interest, and financially impractical. There are always some patients whose condition defies diagnosis. Radioiron studies will usually indicate the type and severity of the physiological defect; that is, a hemolytic process with an average red cell life span of forty days may be indicated, even though the cause for the hemolysis will not. The severity of a given pathological process is often not appreciated with routine tests. For example, in hemolytic disease, a hemoglobin of 10 gm. % does not mean an abnormality with a quantitative ratio of $\frac{10 \text{ gm\%}}{15 \text{ gm\%}}$. More likely, in response to the anemia, hemoglobin production is several times normal, but the hemolytic process is sufficiently severe that even with the increased production a normal level cannot be maintained. Or a normal hemoglobin may be maintained despite the loss of 1% of the total blood volume per day, as a result of twice the normal hemoglobin production. The hemoglobin level would give no indication of the severity of the bleeding.

Combined hematopoietic disorders are likely to give a confused picture with routine diagnostic tests. The iron turnover patterns may also be confusing under these circumstances, but we have had several cases where the correct combination was diagnosed. The quantitative determination of the daily hemoglobin production offers a useful starting point. The half-time and the plasma iron concentration (50, 51, 54, 55) individually often gives an indication of the status of the marrow and of the storage depots.

Additional tests may be performed to further aid in the diagnosis of specific conditions. For example, in iron deficiency anemia, the basic cause may be one

of three:

1. Diet, inadequate in iron or containing chelating substances which bind iron.
2. Inability of the intestinal mucosa to absorb iron.
3. Chronic blood loss.

Possibility 3 is best established by determining the whole blood radioactivity level for some time following a routine turnover test. Possibility 2 can be checked by administering a tracer dose of radioiron sulphate with 5 mg. of therapeutic ferrous sulphate in the early morning, no food having been ingested since a light supper at six the evening before, and food being withheld until noon. Failure of a portion of the administered radioiron to appear in the circulating hemoglobin within 3-5 days is almost certain evidence of inability to absorb iron. Administration of an intravenous iron preparation is the treatment indicated, which may also give additional confirmation of the defect involved. We have encountered one such patient.

Another refinement described by Lawrence et al is designed to differentiate polycythemia secondary to correctable oxygen deficiency from polycythemia vera and uncorrectable decreased oxygenation. An initial half-time is obtained, which is most likely to be rapid. The patient is then placed in an oxygen tent for twenty-four hours. If the polycythemia is secondary to decreased tissue oxygenation as a result of inadequate oxygen transfer across the pulmonary barrier, the oxygen concentration of the blood while in the tent is likely to become normal or excessive. As a result, the stimulus for hemoglobin production will be decreased or eliminated, and the plasma iron clearance rate will be slowed, giving a normal or a prolonged T/2. Patients with polycythemia vera will be unaffected by the rich oxygen atmosphere.

2. Control of hematopoietic therapy: Two main areas are to be considered.---

1. The rapid and quantitative evaluation of administered anti-anemic therapy.
2. The evaluation of treatment for polycythemia by hematopoietic depressants.

The major savings which may result from the early evaluation of therapy directed toward the treatment of anemia, are the time saved for the patient, and the expense

for non-effective drugs. When drugs are effective in altering the rate of hemoglobin production, the change will usually be reflected in the plasma iron clearance rate within twenty-four hours. When the drug in use is shown to be of no value, the patient may be spared the expense of long and repeated trials. The ineffectiveness would otherwise be evident only by failure of the hemoglobin level to show a rise. The conclusion that hemoglobin production is not being improved may take weeks or months to realize when the circulating level is the only criterion. Reticulocyte response may be indicative when the rise is unequivocal, but is subject to more uncertainty and less quantization.

Two examples of early detection of the response to therapy are those of hypoplastic and of pernicious anemia. We have studied four patients with hypoplastic anemia who had failed to respond to routine vitamin therapy. Each had a half-time of between 3 and 5½ hours. As a final trial, each was given large oral doses of cobalt chloride. The half-times of two of these patients changed to less than one hour within twenty-four hours after the initiation of therapy, and they subsequently went on to fair clinical remissions. The other two showed no alteration of half-time at twenty-four hours, and failed to show any improvement after prolonged therapy with cobalt and other hematemic agents.

In pernicious anemia, the half-time becomes even shorter than before treatment within twenty-four hours after vitamin B_{12} administration. The utilization curve changes more drastically, reaching almost 100% in 3-5 days. As treatment continues and the hemoglobin level rises, the half-time and uptake curve gradually approach normal. This will only be true if storage iron is adequate for the production of a normal quantity of hemoglobin.

In the treatment of polycythemia vera with depressant therapy, the problem is more complex. The intent is not to cause the greatest possible change, as in the treatment of anemia; nor is the reticulocyte count of consistent value for evaluating change. Conservative treatment, giving known safe doses of the depressant, whether it be radiation, radioactive phosphorus, or nitrogen mustard,

ordinarily requires a delay of several months until the hemoglobin level stabilizes between successive treatments. Vigorous treatment to reduce this time lag may result in overtreatment and permanent anemia. Determination of the radioiron half-time at appropriate intervals after each treatment allows much more rapid evaluation of the effect of treatment, and therefore less delay between treatments, until a normal hemoglobin production is attained. If single doses of total body radiation are used as the mode of therapy, a slowing of the iron turnover will be evident within twenty-four hours, reaching peak depression at 3-4 days, with some recovery evident during the following one to two weeks. It is well to aim initially at producing a half-time of about 2½ hours, since even with a normal hematopoietic system, hemoglobin production will be depressed by the high hemoglobin level; also the high plasma iron concentration which is maintained for some time by the breakdown of the excess red cells will slow the percentage rate of clearance. When all these phases have reached equilibrium, most patients will show a partial return toward a rapid turnover, and will require retreatment. The second treatment can then be given within a month with little danger of overtreatment.

When radiophosphorus is used for treatment, additional uncertainty is caused by the many weeks over which the radiation is delivered. Radioiron studies will be of little value until about a month after phosphorus administration, and stabilization of the hemoglobin concentration will be correspondingly delayed.

3. Control of hematopoietic damage by depressant agents in the treatment of cancer: Total body radiation, internally administered radioisotopes, and the various chemotherapeutic agents for cancer all cause hematopoietic depression. In our series of 23 patients all treated similarly with 200r skin dose total body radiation delivered from a single anterior field, the plasma iron clearance rate changed in 65% compared to 65% for the white cell elements, and 85% for platelets. Depression in the peripheral white count is rarely pronounced in less than one to two weeks following total body radiation, and the drop in platelet count usually

only starts in two-three weeks, reaching a low in four to seven weeks. The advantage of the plasma clearance rate determination is that the change, when present, is noted within twenty-four hours after the radiation is given with maximal slowing at three days. Recovery of the half-time takes place gradually during the following two to three weeks. The turnover change is temporarily quite severe, an indication that red cell formation is as radiosensitive as is white cell or platelet formation. This would ordinarily not be appreciated, since complete cessation of red cell formation for one to two weeks would not produce a marked change in the peripheral blood count, due to the normal red cell life span of about 120 days. This illustrates one of the advantages of studying the dynamic value of production, rather than the static value of concentration.

In the practice of radiotherapy, the iron turnover test has been of considerable help to us in the evaluation of impending intolerance to total body irradiation (56). For such purpose, the full iron turnover test, with red cell uptake determination, is not as practical an index as is the half-time. For the test to be used to warn of hematopoietic depression from radiation fractionated over several weeks, it is necessary that the test be repeated at periodic intervals; for example, weekly along with the routine hematology. If the plateau in the red cell uptake curve has not been reached by the time of the second test, subsequent uptake percentages will be meaningless. After several tests have been performed, further rise in red cell radioactivity becomes increasingly difficult to quantitate as a percentage of the most recently administered radioiron. On the other hand, the change in disappearance half-time alone appears to be of considerable value. The half-time is reasonably constant over a period of several weeks for any one individual. For example, 28 patients who had repeated turnovers without intervening therapy of any type, and 13 who had small field external radiation without any alteration of peripheral blood counts, showed practically no change in half-time.

Similar changes have been shown following therary with nitrogen mustard and triethylene melamine (57).

Summary

The physiologic background and rationale for the clinical application of radioiron tracer studies has been presented, along with detailed directions for the performance of these studies. Interpretation of the data is discussed. Over 700 turnover studies on more than 300 patients from our department have been analyzed to present a table of normal and abnormal values for the hematopoietic disorders studied.

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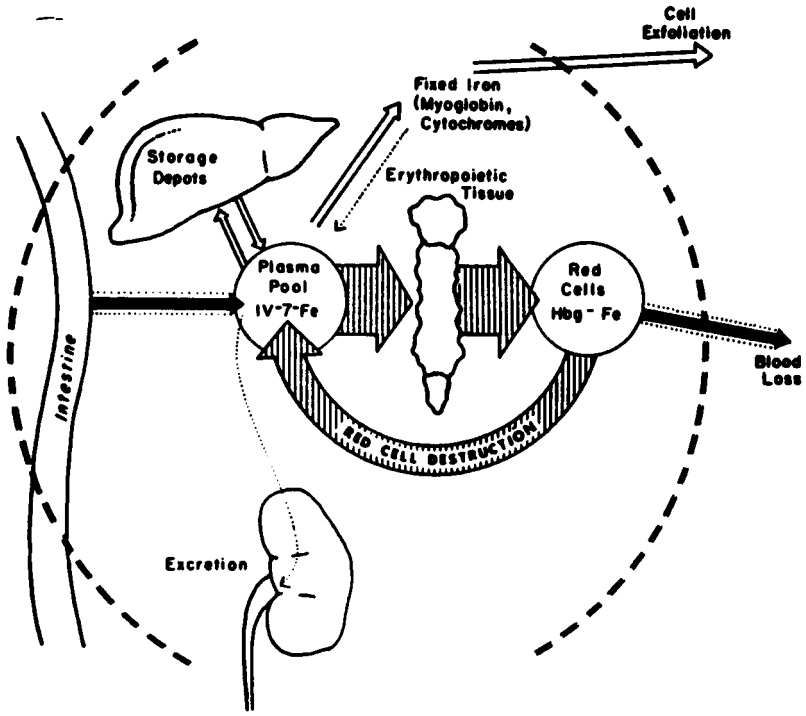


FIGURE I
NORMAL IRON METABOLISM

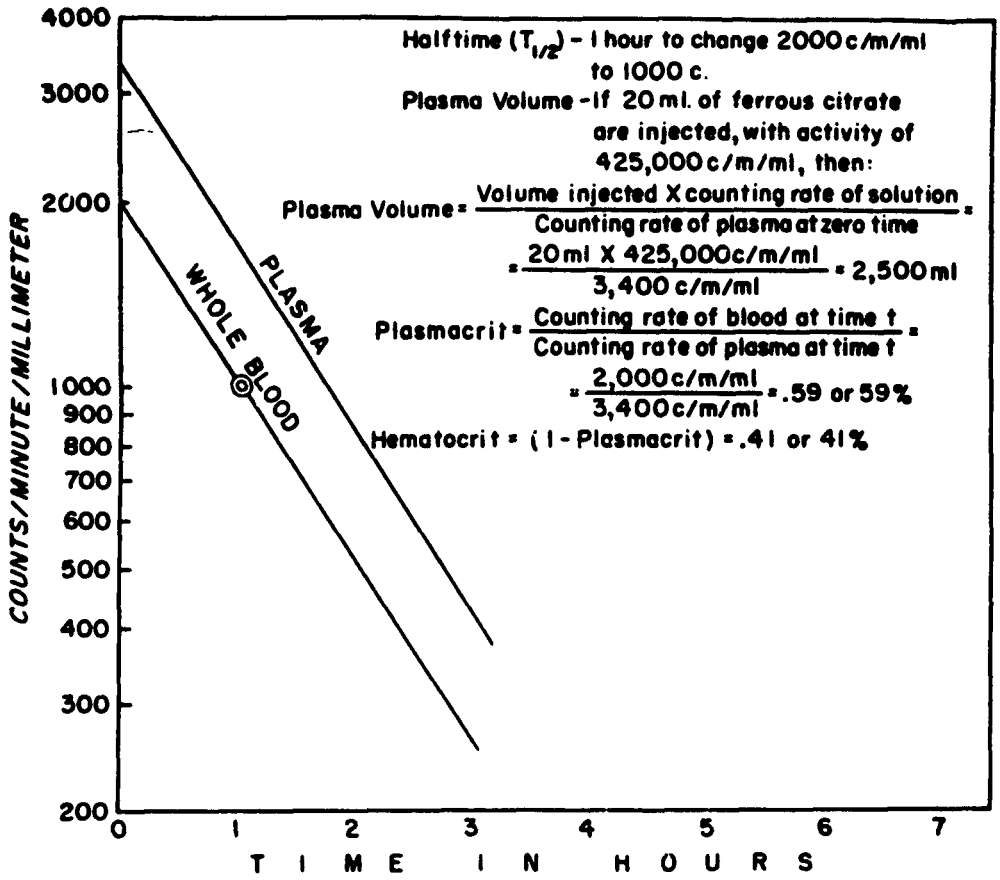
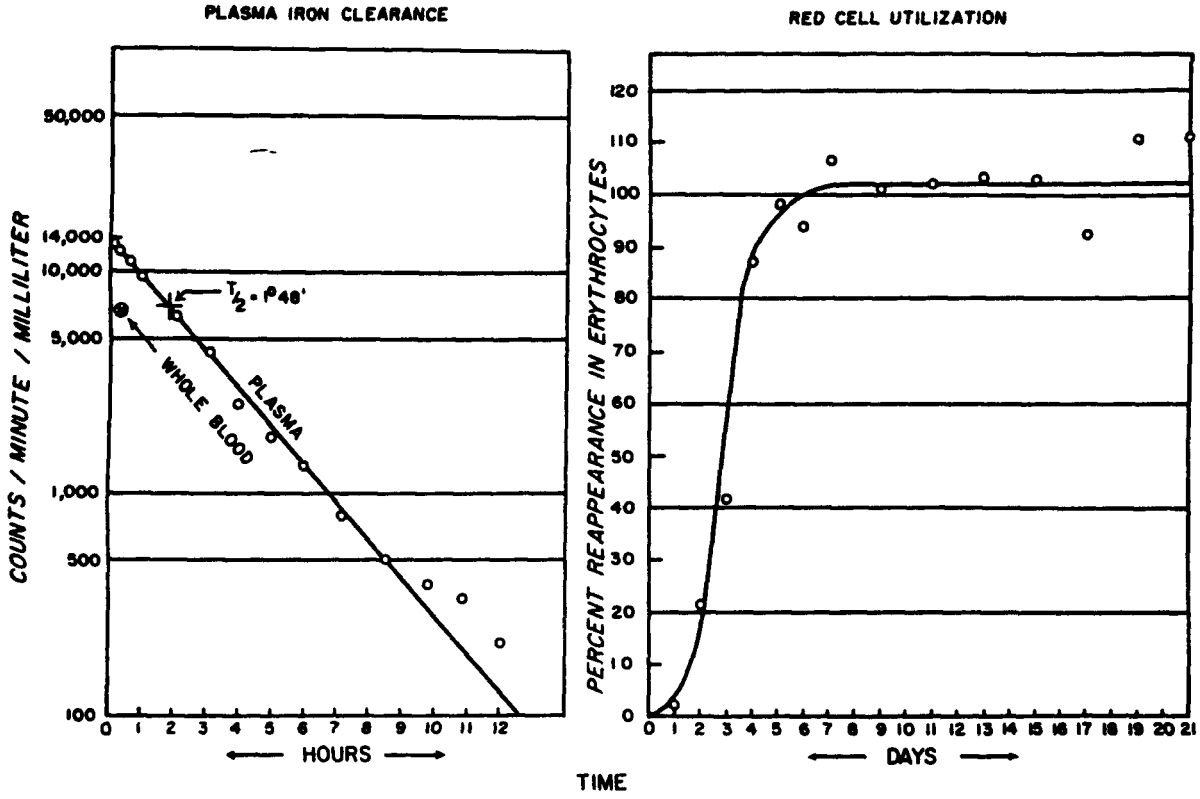

Halftime ($T_{1/2}$) - 1 hour to change 2000 c/m/ml to 1000 c.
Plasma Volume - If 20 ml. of ferrous citrate are injected, with activity of 425,000 c/m/ml, then:
Plasma Volume = Volume injected X counting rate of solution / Counting rate of plasma at zero time =
= 20 ml X 425,000 c/m/ml / 3,400 c/m/ml = 2,500 ml
Plasmacrit = Counting rate of blood at time t / Counting rate of plasma at time t =
= 2,000 c/m/ml / 3,400 c/m/ml = .59 or 59%
Hematocrit = (1 - Plasmacrit) = .41 or 41%
PLASMA
WHOLE BLOOD
COUNTS/MINUTE/MILLIMETER
4000
3000
2000
1000
900
800
700
600
500
400
300
200
0
1
2
3
4
5
6
7
TIME IN HOURS

Figure II

FIG.III : RADIOIRON TURNOVER STUDY ON THE AUTHOR



APPENDIX #2

Method for Determining the Iron-Binding Capacity of Serum

by

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The availability of radioactive iron has permitted a laboratory evaluation of hematopoietic disorders based upon the rate and quantity of iron transport by the serum, and the subsequent distribution of this iron (1,2,3). To interpret the data obtained from iron turnover studies, it must be known that the serum transport mechanism is not the limiting factor, by demonstrating residual iron-binding capacity. The serum iron concentration and residual binding capacity of normal persons and of patients with a variety of diseases have been determined using several published chemical and isotopic procedures (4-15). The simplicity of the method described here for analyzing for serum iron-binding capacity has permitted this laboratory to obtain reproducible values on a large number of samples. Iron labelled with a radioactive tracer is added to a serum sample in excess of the binding capacity, and that which becomes attached is separated by protein precipitation and measured isotopically.

Preliminary studies indicated several potential pit-falls. Ionic iron will not remain in solution at neutral pH, but precipitates as ferric hydroxide which has a $k_{s.p.}$ of 1.1×10^{-36} ($18^{\circ}C$). Several authors have advocated addition of iron chloride in acid solution directly to serum to obtain protein-bound iron, since the buffering capacity of serum is adequate to neutralize small volumes of these weakly acid solutions. If this is done, the iron is not dialysable, and precipitates with the protein fraction. Nevertheless, much of the added iron

is in the hydroxide form, and will not permit determination of iron-binding capacity, nor be a tracer for protein-bound iron in vivo. Iron complexes which are stable at neutral pH may be used, but the stability of the complex must not be sufficiently great as to inhibit iron from transferring to the iron-binding protein fraction (IV-7, Cohn). A subsidiary study indicated that iron will distribute between equal quantities by weight of citrate and of iron-binding protein in a ratio of one to seventy. Gluconate binds iron somewhat more firmly than does citrate, and versenate has even greater affinity than does the protein. The most satisfactory agent was found to be ascorbic acid in small concentration.

Ammonium sulfate is the precipitating agent of choice (11). The precipitated protein colloidal suspension is easily broken by ethanol, facilitating separation by centrifugation. Small amounts of the filtrate are occluded in the precipitate during centrifugation. A washing procedure is not necessary, however, since isotope studies indicated that less than 0.1 ml containing less than 4% of the total excess iron in the filtrate is entrapped. Filtration is laborious and time consuming, and often subject to errors of adsorption.

Heparinized plasma cannot be used in place of serum, as there is apparently an interference with iron transfer causing false low iron-binding capacity

values to be obtained.

METHOD

A. Reagents:

(1) Carrier-radioiron chloride solution: $Fe^{59}Cl_3$ in HCl is diluted to approximately 0.2 uc/ml. Reagent grade $FeCl_3 \cdot 6H_2O$ is dissolved in the solution to give a total iron concentration of about 60 ug/ml, and standardized against a solution made from iron wire dissolved in HCl. This solution may be stored in polyethelene bottles. If iron wire dissolved in HCl is used instead of $FeCl_3$ crystals, the total acidity of the final reagent (solution #2) must not exceed the buffering capacity of the serum.

(2) Carrier-radioiron ascorbate solution: About 10 mg of pure crystalline ascorbic acid is dissolved in 1.0 ml of solution #1 just prior to use.

(3) Saturated ammonium sulfate solution: 530 gm of reagent grade $(NH_4)_2SO_4$ (iron not in excess of .0001%) is dissolved in almost one liter of iron-free water, the pH adjusted to 6.0 by addition of NaOH solution, and brought to a final volume of one liter. The pH of this solution should be checked occasionally as it may become lowered due to loss of NH_3 on long standing.

(4) 95% ethanol.

B. Procedure: All glassware is obtained new and maintained free from iron. It is washed with a good detergent, rinsed thoroughly in tap water, and finally rinsed twice with distilled water.

Steps:

1. Measure 0.1 ml carrier radioiron ascorbate solution into a pyrex test tube 15 x 125 mm.

2. Add 1.0 ml serum and mix well. Allow to stand for 10 minutes at room temperature and count the radioactivity.

3. Add 10.0 ml of $(NH_4)_2SO_4$ solution, mix by shaking, and allow to stand for one hour.

4. Add 1.0 ml ethanol, invert several times and centrifuge for ten minutes at about 2500 rpm.

5. With a fine glass stirring rod, gently tilt the protein plug and while holding it against the wall of the tube, carefully pour off supernatent liquid. Push the plug to the bottom of the tube and loosen any precipitate adherent to the walls of the tube with the stirring rod and rinse down the rod and wall of the tube with 1.0 ml distilled water.

6. Count the precipitate and calculate the iron-binding capacity according to the formula:

$$\frac{\text{Counts of the precipitate}}{\text{Original counts added}} \times \text{ug Fe added} \times 100 = \text{ug \% binding capacity}$$

The method was evaluated as follows:

1. Tagged iron was added to one ml samples of pooled patient sera in successive increments of 0.5 ug, allowed to incubate 10 minutes after which 6.0 ug of cold iron as ascorbate was added, and the radioiron in the precipitated protein of each was measured. Results of this experiment show that the radioiron added to the unsaturated protein remains attached during the test (Table I).

2. Pooled patient sera was saturated with cold iron ascorbate, and radioiron ascorbate added secondly to prove that exchange does not take place between bound iron and unbound iron (Table II).

3. Graded increments of cold iron ascorbate were added to pooled sera samples, and residual iron-binding capacities measured (Table III).

Both serum iron concentration and iron-binding capacity can be determined on the same one ml sample. The iron-binding capacity is determined as above. The total iron in the tube is then determined chemically. The initial serum iron concentration is the difference between the total iron and the iron-binding capacity values.

The amount of iron in the $(NH_4)_2SO_4$ solution is approximately 150 ug %. Less than 0.1 ml remains in the tube with the protein precipitate, this additional iron being negligible. Thus, if cold iron were used in the first

step, a modification of the procedure after step 5 would permit chemical, rather than isotopic analysis. The modification would consist of determining the total iron concentration in the tube containing the suspended protein precipitate, as well as the original serum iron concentration using a separate one ml sample. By difference, the iron-binding capacity could be calculated. This method would be of value in a laboratory not equipped to use radioisotopes.

TABLE I

Recovery of Added Radioiron

Tube	Fe* ascorbate added to serum (ug of Fe)	Fe* measured in protein plug (ug of Fe)
1	0.5	.47
2	1.0	.93
3	1.5	1.40
4	2.0	2.03
5	2.5	2.30*
6	5.0	2.37*

*Serum saturated.

TABLE II

Results of adding Fe* to serum previously saturated with Fe

Tube	Fe* ascorbate added to saturated serum (ug of Fe)	Fe* measured in protein plug (ug of Fe)
1	0.5	0.04
2	2.5	0.18
3	5.0	0.26

TABLE III

Results of Iron-Binding capacity measurements following the addition of various amounts of cold iron

Tube	Fe added to serum ug %	Residual Fe-binding capacity ug %	Difference in Fe-binding from original ug %
1	0	296	
2	50	250	46
3	100	193	103
4	150	143	153
5	200	90	206
6	250	50	246
7	500	22	274

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APPENDIX #3

Radioiron Tracer Studies for the Evaluation of Radiation Damage

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Major interest in the use of radioiron tracer studies to evaluate radiation damage to the erythropoietic system was stimulated by the work of Hennessy and Huff (9). They were able to demonstrate a decrease in the rate and percent of incorporation of injected radioiron into red cells in individual rats which had received 25 r or more of total body radiation. Statistically, groups of rats having received as little as 5 r could be differentiated from unirradiated rats. The severity of depression is dependent upon the radiation dose, at least up to 250 r (Figure I). Belcher et al (2) have confirmed these results. The present studies were intended to assess the usefulness of one phase of the radioiron turnover test as an adjunct to routine peripheral blood counts in clinical radiotherapy.

Hennessy's data indicates that erythropoietic tissue is as radiosensitive as are the tissues producing white cells and platelets. Because of the life span of the normal erythrocyte however, complete cessation of production will not appreciably affect the circulating hemoglobin level for one to two weeks. The purpose of the complete radioiron turnover test is to determine the rate of hemoglobin production, permitting appraisal of daily erythropoietic function (11,25).

These tracer studies should be considered in relation to normal iron metabolism (Figure II). The iron in the plasma is undergoing continuous turnover in a reasonably steady state system. Most of the iron entering the plasma pool is that released from destroyed red cells with lesser contributions from storage depots and from the digestive system. Eighty to ninety percent of the iron leaving the plasma is taken up by the erythropoietic tissues, the rest going to storage depots, to growing tissues for utilization in the cytochrome enzyme systems, and several milligrams per day being excreted in feces, urine, and sweat. The normal plasma iron concentration is about 0.1 mg per 100 ml of plasma. The normal red cell life span being about 120 days, each 100 ml of plasma has to transport about 1 mg of iron per day to the bone marrow for hemoglobin production. Thus it is

evident that the plasma iron must be replaced about 10 times per day. The iron concentration alone does not permit estimation of the turnover rate. By injecting a radioactive tracer into the vascular system and determining the activity of successive blood or plasma samples, the clearance rate can be measured (24). This radioactive tracer follows the same metabolic pathways as does the native plasma iron present at the time of injection. A reasonably constant portion is cleared by the erythropoietic tissues, the liver and other storage depots, and miscellaneous tissues with each circulation. While non-radioactive iron enters the circulation to replace that being removed, the radioactive component is continually being depleted. Since a constant fraction is cleared with each circulation, the decrease in activity is exponential; the rate of decrease may be defined by the time required to reach half of the initial activity---the half-time. Plasma iron clearance is calculated from the equation:

$$\text{Micrograms iron cleared per hour per 100 ml plasma} = \frac{0.693 \times \text{plasma iron concentration in micrograms \%}}{\text{Half-time of disappearance in hours}}$$

The hourly clearance of iron from the entire plasma pool is calculated from the clearance per 100 ml and the plasma volume. The plasma volume is obtained from the initial dilution factor of the injected tracer, as part of the clearance rate test. The daily clearance is approximately 24 times the hourly clearance, but is subject to uncertainty due to diurnal changes in iron concentration, plasma volume, and half-time (3,20). Since a variable portion of the iron leaving the plasma is incorporated into red cells, this percentage must be experimentally determined in order to calculate daily hemoglobin production. Whole blood samples are analyzed for radioactivity until a plateau is reached, usually at 5-7 days, and total red cell radioactivity expressed as percent of injected activity. Hemoglobin production is calculated from the equation:

Hemoglobin produced per day (grams) =

$$\frac{16.6 \times \text{plasma vol. (ml)} \times \text{plasma iron conc. (mg/ml)} \times \text{percent utilization}}{\text{Disappearance half-time (hours)} \times 3.34 \text{ (mg/gm hgb)}}$$

Because of the interdependence of the four variable factors, some authors report only the computed daily hemoglobin production. While the level of hematopoietic activity is not determinable from any of the variables independently, such condensation fails to utilize the available data to the maximum extent (6-8, 13,14,18,19,27,29). Over 700 iron turnover tests have been performed by this department in the past three years as a laboratory routine for the differential diagnosis of hematopoietic disorders (25). The observed values for some conditions are presented in Table I. In general, half-times of less than normal are observed when anemia is present despite adequate erythrogenic activity, and prolonged half-times when anemia is on a hypoplastic basis.

The usefulness of radioiron tracer studies for evaluation of radiation damage results from the quantitative assessment of induced hypoplasia. Decreased erythropoietic activity is usually characterized by long half-time, high plasma iron concentration, and poor utilization of the cleared iron. The animal studies of Hennessy and Huff correlate the last variable with dose of radiation.

These workers have also used the change in plasma iron clearance half-time as an index of marrow depression by radiophosphorus in the treatment of polycythemia vera (11). The clearance rate would be a more satisfactory variable than the percent uptake to use as a therapeutic control, if equal usefulness could be established. The clearance rate can be determined in 1-3 hours. Since the plasma is cleared of significant activity within 24 hours, tests can be repeated daily. The percent uptake cannot be dependably determined until a plateau in the red cell radioactivity has been reached, which may take from 5 to 7 days. If a second tracer is given before a plateau has been obtained from the first, it is impossible to determine

the percent incorporation of each. After several radioiron studies, the red cell radioactivity becomes sufficiently high that additional activity is difficult to determine accurately. This difficulty can be overcome by administering successively larger tracer doses, but the acceptable human tracer burden is soon reached. A serious disadvantage of the half-time alone is that the site of clearance is not indicated. As erythropoietic activity is depressed, an increasing portion of the plasma iron is cleared by the liver. The present studies were conducted to determine whether this uncertainty negates the value of the test as an adjunct to routine blood counts for the evaluation of systemic tolerance in the practice of radiotherapy.

The study had three phases:

1. Correlation of change in half-time with change in peripheral hematology and with symptomatology in patients receiving therapeutic levels of total body radiation.

2. Similar comparison in patients receiving radiation through limited portals.

3. Investigation in rabbits of an extended procedure using turnover studies to detect residual hematopoietic damage at longer time periods following total body radiation, when the peripheral blood levels have returned to normal and the individual has apparently returned to pre-irradiation status.

Investigational

I. Sixteen persons, including staff personnel with apparently normal hematopoietic systems and patients with a variety of disorders not directly related to the hematopoietic system, had two or more determinations of their plasma iron clearance rates at varying intervals. The variation between any two half-times on the same individual was less than 30% in eighty-two of the eighty-eight possible comparisons. When summarizing subsequent data, change of less than 30% was not considered significant.

Thirty patients with generalized neoplastic disease received total body

radiation in doses ranging from 100 to 200r in single exposures, and from 250 to 550r total when fractionated (5,23). Dose is described as skin dose to the mid-surface of the patient, the radiation factors being 250 KVP, HVL of 1.2-2.3 mm Cu, TSD of 360-400 cm. Changes in half time after irradiation for ten of the patients who received 200r to the anterior body surface are shown graphically in Figure III. Daily variation in peripheral blood counts and in radioiron half-time of three of these patients are shown individually in Figures IV-VI. The last of these shows the changes following two separate doses of 200r each, separated by five months. Data on the thirty patients is summarized in Table II, grouping patients with approximately equal radiation exposure. The plasma clearance rate is altered about as frequently as is the platelet and white cell count. Change in laboratory data is more frequent than is symptomatology in this dose range. Change in clearance rate occurs more promptly than does change in blood count and shows more rapid return to normal. Some patients had alteration in only one or two of the functions studied.

II. Thirty-eight patients received fractionated radiation therapy through limited portals. The percentage change in half-time for each is shown in Figure VII, plotted as a function of energy absorbed. The plasma iron half-time was determined within three days prior to the beginning of therapy, and again on the last or next to last treatment day. Integral dose is calculated as described elsewhere (26). No attempt has been made to compensate for the dependence of effect upon both time and dose, since there are no useful factors available which would permit meaningful adjustment of stated values to a common baseline. Since most patients were treated over a three to five week period, such adjustment would likely be of little importance. No correlation is evident between integral dose and slowing of plasma clearance rate. Grouping the patients by region treated does not improve the correlation.

Laboratory data is given in Table III on two patients with seminoma who received a mid-point tissue dose of 1500r in three weeks to the lymphatic

drainage areas of the pelvis and abdomen, through opposing anterior and posterior fields totalling 500 square centimeters to each side. Plasma clearance rates were determined at weekly intervals. The half-time of each slowed at first, then recovered as treatment continued. These changes may reflect an initial depression of marrow function within the treated volume, followed by compensatory hyperfunction of unirradiated erythropoietic tissues. The white count continued to decline during treatment, consistent with the decrease in residual tolerance as absorbed dose increases. Possibly an occasional patient would show a dramatic slowing of the plasma iron clearance rate without subsequent recovery, as an indication that erythropoiesis will limit the tolerable dose. The data given suggests that this will occur infrequently if at all with local irradiation.

III. A preliminary experiment was conducted, intended to develop a method for measuring residual hematopoietic damage. Most organs of the body are able to increase their functional level when the need arises, although this reserve capacity is not usually evident or measured. Of two persons, each with a normal hemoglobin concentration, it may be that one could greatly increase hemoglobin production in response to a demand created by bleeding, hemolysis, or hypoxia; while the other is just barely able to maintain a normal level, and could not increase erythropoiesis even should the need arise. Prior to the stimulus for increased production, routine hematological examination would not reveal any difference between the two persons. A decrease in reserve capacity could partially explain the greater response to a second equal dose of radiation which is usually observed even when no residual damage from the first dose is apparent. Patient F. M. illustrates this increased response to total body radiation (Figure VI). This patient had generalized lymphosarcoma, but was otherwise in good health. He was given 200r skin dose to his anterior body surface, radiation factors being 250 KVP, HVL of 1.2 mm Cu,

TSD of 400 cm. He developed no symptoms, and no significant change in white count or plasma clearance rate. The platelet count remained within normal limits despite a late drop. The generalized lymphadenopathy disappeared, and the patient had no gross evidence of disease for five months, when the lymphadenopathy returned. Findings on physical examination and peripheral blood study were similar to those of five months previously. Yet a second dose of 200r caused nausea and vomiting for 3 days with considerable change in white and platelet counts and plasma iron clearance rate.

If loss of reserve capacity of various body systems does explain some of the residual damage from radiation, it should be possible to demonstrate the defect by presenting a stimulus to increased activity of a system, and observing an inadequate response. Rabbits were chosen for study because they are just large enough to permit repeated venipunctures and are available in reasonably homogeneous strains. Change in plasma iron clearance rate was used as an index of alteration in function. Hypoxia was adopted as a stimulus (12,21,22), the rabbits being maintained in a decompression chamber at 2/3 atmosphere. The standard radiation dose was 500r surface dose, 250 KVP, HVL-1.2 mm Cu, TSD of 100 cm, delivered from above to the back of each rabbit in a 40 x 40 cm box filled with rice bolus. Clearance rate determinations were limited to two per rabbit, since significant blood loss would act as a stimulus similar to hypoxia, with additional changes possibly resulting from removal of other blood elements. The plasma iron clearance rate of each animal was determined initially, and again at varying times after being subjected to one of the three experimental conditions.

The first group was given 500r total body radiation. Each rabbit had a second half-time determination within two weeks following radiation. In Figure VIII is shown the change in half-time with time after radiation. Each point on the curve represents ten rabbits. The shaded area represents fewer animals, and demonstrates the variability in time required for the

half-time of individual rabbits to return to normal.

The second group was placed in the decompression chamber at 2/3 atmosphere for from one to five days. As shown in Figure IX, the iron clearance half-times became shorter. The half-time changes were quite variable until the third day, after which almost all rabbits had half-times of thirty to forty minutes.

The third group was irradiated to 500r, and after varying periods of time each rabbit was placed in the decompression chamber for three days. The second clearance rate determination was performed immediately after removal from the chamber. Eighteen rabbits were used in the preliminary phase of this experiment. As shown in Figure X, the results were not sufficiently consistent to warrant repetition with a larger group of rabbits under the same experimental conditions. Rabbits which were irradiated and immediately placed in the decompression chamber for three days, had slow half-times similar to those of rabbits receiving radiation alone. This was expected, as it was considered unlikely that excess stimulus could overcome the damage caused by radiation. Several of the rabbits maintained at atmospheric pressure for a week or longer after irradiation, then subjected to three days of hypoxia prior to the second clearance rate determination, demonstrated the inadequate response to hypoxia which was anticipated. However, some rabbits having had only five to nine days to recover from 500r, already showed a normal response to the erythropoietic stimulus. This data is not sufficient to either prove or disprove the concept of decreased functional reserve as a radiation residuum. It is certainly too inconsistent to suggest a potential clinical test.

Discussion

Radioiron tracer studies are of clinical value for the diagnosis of anemias whose etiology is not completely apparent from less esoteric examination; and for the diagnosis and control of therapy of polycythemia vera. As a research tool, radioiron has been used as a tracer for the elucidation of various

pathways and mechanisms of iron metabolism; for the quantitative evaluation of the effects of whole body radiation in animal species; for the study of the mode of action of radiation upon the erythropoietic system; and for the appraisal of agents which may stimulate or depress erythropoietic function (1,15-17,28).

In the initial studies on rats by Hennessy and Huff, iron was injected at varying times following irradiation and the rate of red cell incorporation determined. The best correlation between dose of radiation and reduction of incorporation was noted when the iron was injected twenty-four hours after irradiation. Greater time delay resulted in varying degrees of recovery, the rate of recovery also being dependent upon the dose administered. With doses of less than 200r, the initial depression for several days was followed by a return past normal to an increased utilization for several days (10). This overcompensation may be due to the stimulus of the slightly decreased hemoglobin resulting from the period of depressed hematopoietic activity.

The relationship between dose of radiation and depression of iron utilization is more nearly exponential than linear, with successively larger doses being required to cause additional equal depression. It has been postulated that this relationship indicates direct damage to the red cell precursors, inhibiting their ability to divide and produce new red cells. An alternative explanation would be that radiation blocks one or more of the reactions involved in hemoglobin formation, by direct inhibition of some enzymes. Evidence for the direct effect of radiation on red cell precursors is provided by studies of Lejtha and Suit (17). These workers investigated the utilization of radioiron by bone marrow cultures using radioautographic techniques, and noted that iron was incorporated into the developing cells primarily during the late pronormoblast and basophilic normoblast stages. When the marrow cultures were irradiated, and iron incorporation studied at successive intervals thereafter, it was noted that the accumulation of radioiron by cells of a given developmental stage was not impaired. Immature

cells continued to progress toward more mature forms, but no new cells were produced. A gradually shifting cell population resulted. Within several days after radiation, cells at the iron-accepting level of development were almost entirely absent, and iron incorporation by the culture practically ceased. These studies provide direct evidence on the mechanism of radiation damage, which explains our in vivo observation that maximal depression of the plasma iron clearance rate occurs about three days after total body irradiation in humans and in rabbits. The failure of the plasma iron clearance rate to change in proportion to the dose of partial body radiation is also consistent with a direct effect of radiation upon marrow cells. Hennessy (10) studied the utilization of iron by rats having received varying doses to the abdomen from sharply defined beams of deuterons, positioned to avoid all bone marrow. Even though the rats showed other radiation effects, none, even those having received a lethal dose, showed change in iron utilization.

Radioiron tracer studies have been shown to be of value for the evaluation of erythropoietic depression following the administration of systemic agents and deserve more widespread use. John H. Lawrence and co-workers have already established the usefulness of the test to control the treatment of polycythemia vera by radiophosphorus. That most chemotherapeutic agents used for the treatment of patients with cancer are hematopoietic depressants is accepted; that such depression is reflected in the iron turnover test has been shown. The intent of treatment is usually to cause the greatest possible controlled destruction from which the patient can satisfactorily recover. Any additional examination or test which can help establish this limit should be utilized whenever possible.

The problem of measuring residual radiation damage may become one of major importance. At the present time, only a relatively few persons are exposed to radiation, usually in a manner which is controllable and can be

held to an insignificant level. With the likely increase in the number and types of nuclear reactors, it is possible that a much larger portion of the population will join the chronically exposed group. It may be that the further economic development of the country will some day necessitate a relaxation of the present permissible tolerance levels. Should this necessity arise, possible radiation damage may become accepted as an industrial hazard with a known calculated risk, similar to silicosis in miners, or the possibility of falling among construction workers. A method for measuring damage before it becomes grossly evident and irreversible would then be of value. The leukopenia and anemia which is occasionally observed in persons having received prolonged chronic exposure are apparently almost irreversible. This is consistent with the concept of a gradual depletion of reserve functional capacity. Even though the experiment reported above failed to confirm this concept, the conditions chosen may not have been adequate.

Should the basic concept prove useful, other methods of demonstrating the residual radiation defect will have to be found. To spend several days in a decompression chamber is feasible as a research procedure, but not practicable as a routine for thousands or millions of persons. Possible alternatives are apparent. There is some evidence of a circulating hormone which stimulates erythropoiesis. Should this hormone be isolated, it could serve in lieu of hypoxia as a maximal stimulus. Other radiosensitive tissues or functions other than erythropoiesis should be studied. A variety of insults to the body cause a leukocytosis. Perhaps a reasonably quantitative response to an injection of dead bacteria could be established as a test system. The production of hydrochloric acid by the gastric mucosa is depressed by acute radiation exposure. If this function is also depressed by chronic low-level exposure, the alcohol-histamine test is already available as the stimulus for maximal production. It is disappointing that this study was unsuccessful, but the problem of residual radiation damage is considered of

sufficient importance to warrant further efforts.

Summary

The plasma iron clearance rate was determined during and following treatment in cancer patients receiving total body and limited field irradiation. Slowing of the clearance rate as an index of erythropoietic depression, was compared with radiation dosage, symptomatology, and other laboratory evidence of systemic depression. This data suggests the usefulness of radioiron tracer studies on a routine basis for early indication of impending intolerance to generalized therapy for cancer. No useful correlation was observed with limited field irradiation.

Extension of radioiron tracer studies to provide a test for residual radiation damage is described and discussed, even though a preliminary experiment failed to indicate a useful technique.

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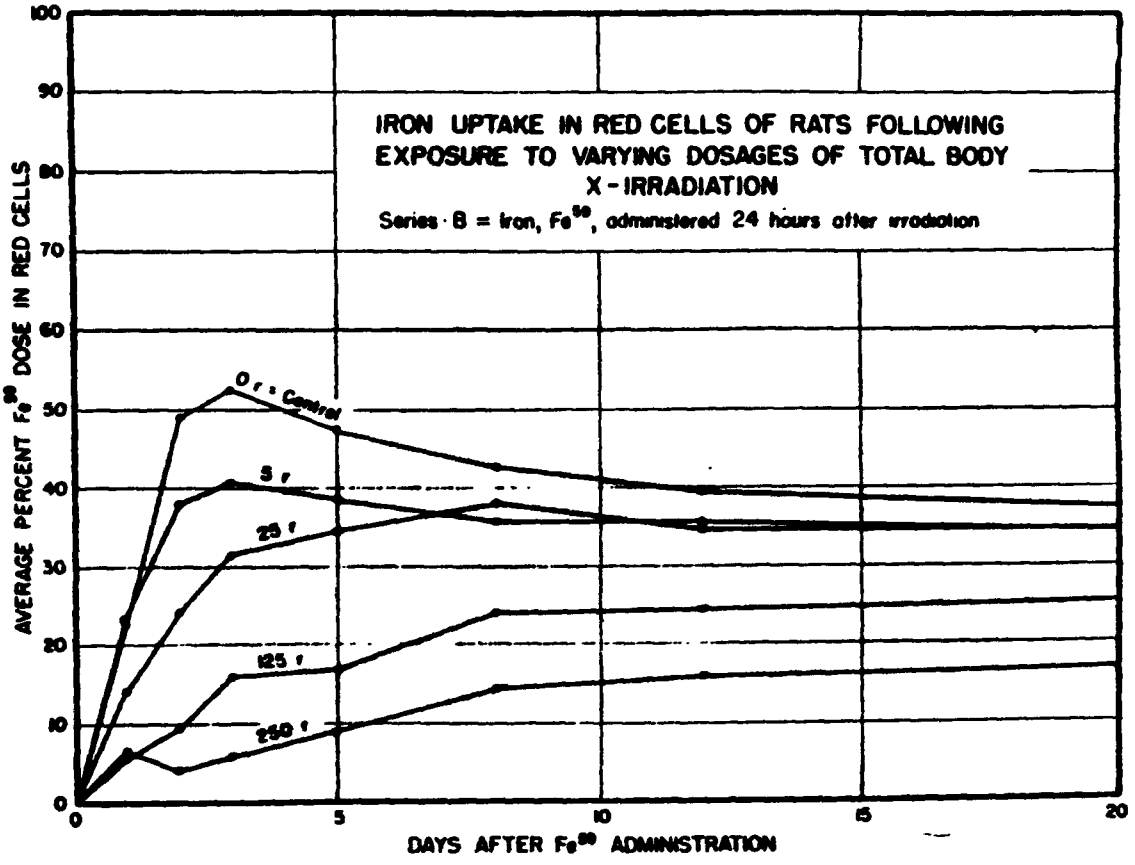
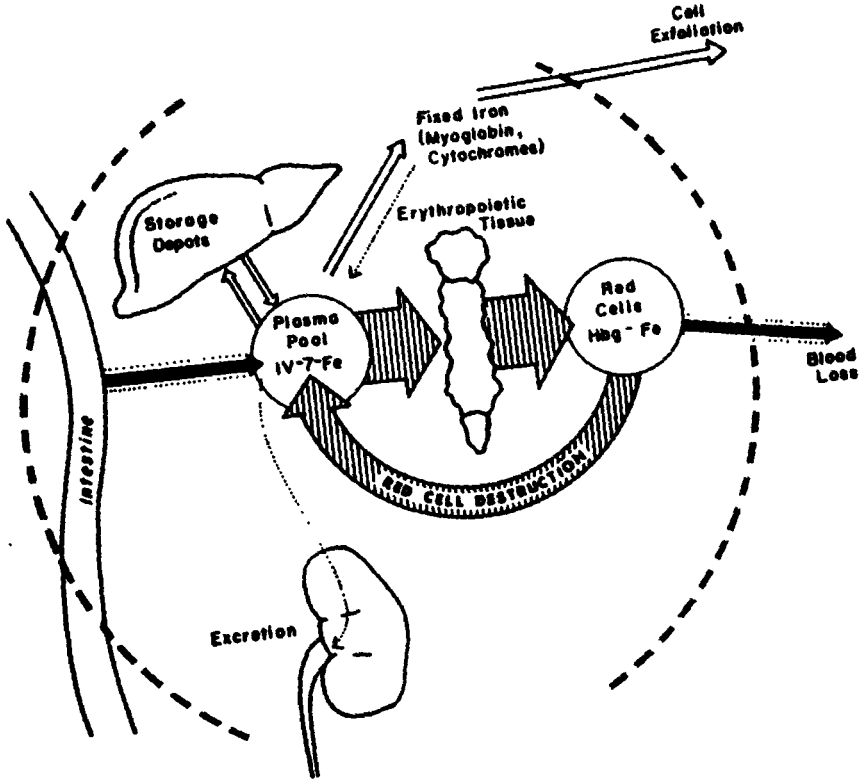

IRON UPTAKE IN RED CELLS OF RATS FOLLOWING EXPOSURE TO VARYING DOSAGES OF TOTAL BODY X-IRRADIATION
Series B = Iron, Fe59, administered 24 hours after irradiation
AVERAGE PERCENT Fe59 DOSE IN RED CELLS
DAYS AFTER Fe59 ADMINISTRATION
0 r = Control
5 r
25 r
125 r
250 r
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20

Figure I



NORMAL IRON METABOLISM

Figure II

HEMATOLOGICAL CONDITION	FEP-C	T/2	UPTAKE
	UG %	HOURS	%
NORMAL	60-110	1-2	UP TO 90% IN 1 WEEK. THEN PLATEAU.
ANEMIA			
IRON DEFICIENCY	0-50	0.3-0.5	APPROACHING 100% IN 3-4 DAYS. THEN PLATEAU.
CHRONIC BLOOD LOSS	0-50	0.3-0.5	APPROACHING 100% IN 3-4 DAYS. THEN STEADY FALL OFF.
HEMOLYTIC	40-70 (OCC. HIGH)	0.3-0.5	APPROACHING 100% IN 3-4 DAYS. THEN PLATEAU.
CHRONIC INFECTIONS) MALIGNANT TUMORS) LEUKEMIAS) LIVER DISEASES)	40-70	0.3-1	APPROACHING 100% IN 3-4 DAYS. THEN PLATEAU.
APLASTIC	150-300	2-6	VERY LOW AND SLOWLY RISING.
ADDISONIAN			
UNTREATED	150-200	0.3-0.6	SLOW RISE OVER MANY WEEKS, NO PLATEAU.
REMISSION			
EARLY	40-70	0.3-0.4	APPROACHING NORMAL.
LATE	60-110	1-2	
MEDITERRANEAN	150-200	0.3-0.6	SLOW RISE OVER MANY WEEKS, NO PLATEAU.
POLYCYTHEMIA			
PRIMARY, UNTREATED	50-100 (OCC. HIGH)	0.3-0.5	APPROACHING 100%.
REMISSION	60-110	1-2	NORMAL.
REPEATED PHLEBOTOMY	40-70	0.3-0.5	APPROACHING 100%.
SECONDARY	50-100 (OCC. HIGH)	0.3-0.5	APPROACHING 100%.
HEMOCHROMATOSIS	200-250	1-2	LOW UPTAKE.

Table 1

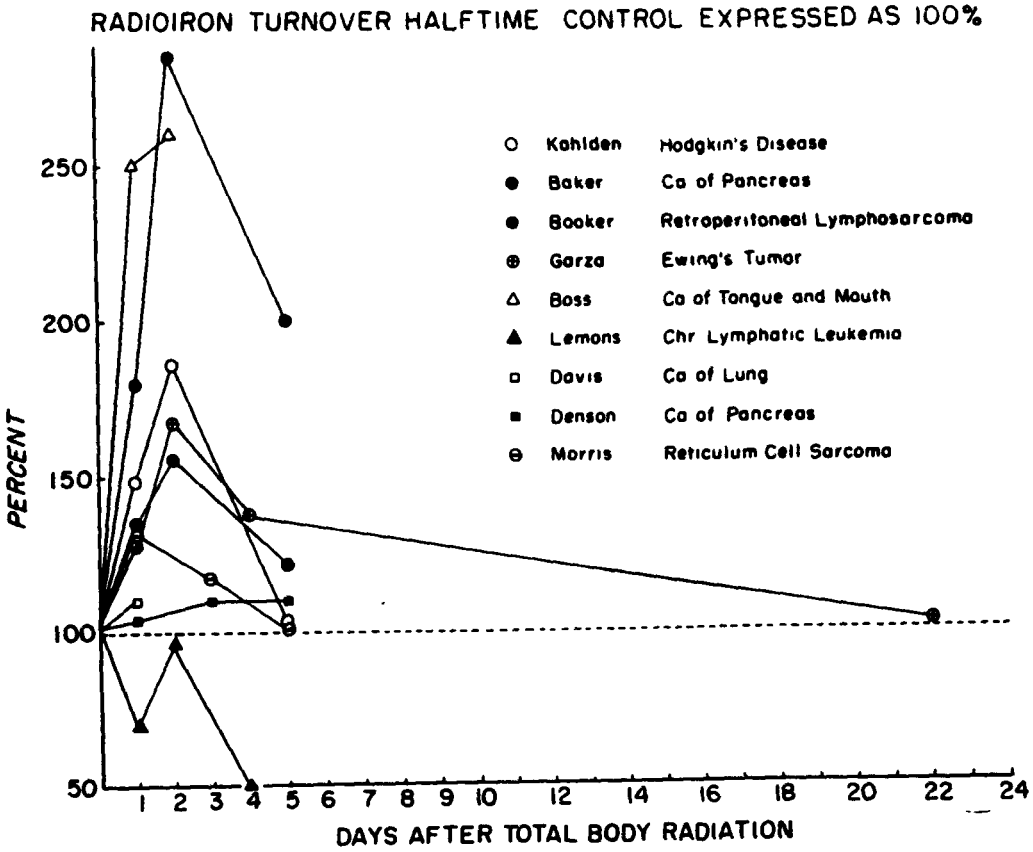

RADIOIRON TURNOVER HALFTIME CONTROL EXPRESSED AS 100%
PERCENT
250
200
150
100
50
1 2 3 4 5 6 7 8 9 10 12 14 16 18 20 22 24
DAYS AFTER TOTAL BODY RADIATION
Kahlden Hodgkin's Disease
Baker Ca of Pancreas
Booker Retroperitoneal Lymphosarcoma
Garza Ewing's Tumor
Boss Ca of Tongue and Mouth
Lemons Chr Lymphatic Leukemia
Davis Ca of Lung
Denson Ca of Pancreas
Morris Reticulum Cell Sarcoma

Figure III

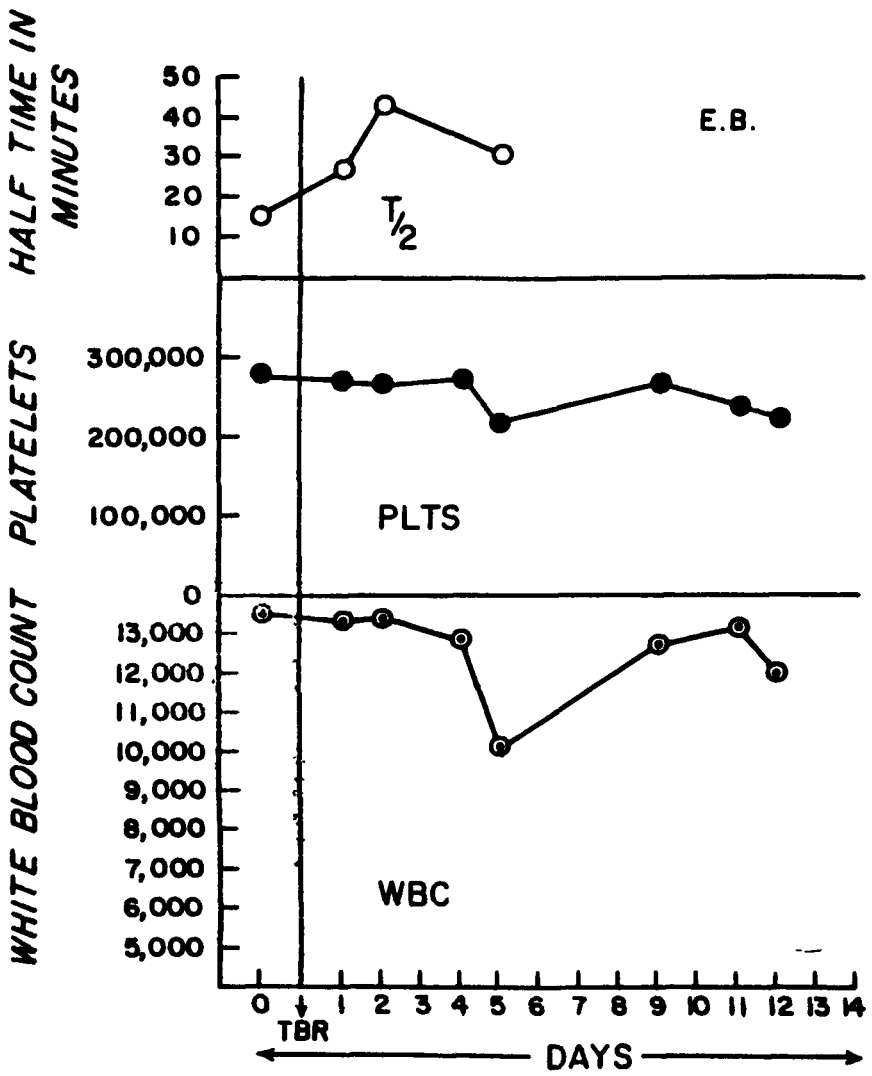

HALF TIME IN MINUTES
PLATELETS
WHITE BLOOD COUNT
50
40
30
20
10
E.B.
T/2
300,000
200,000
100,000
PLTS
0
13,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
WBC
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
TBR
DAYS

Figure IV

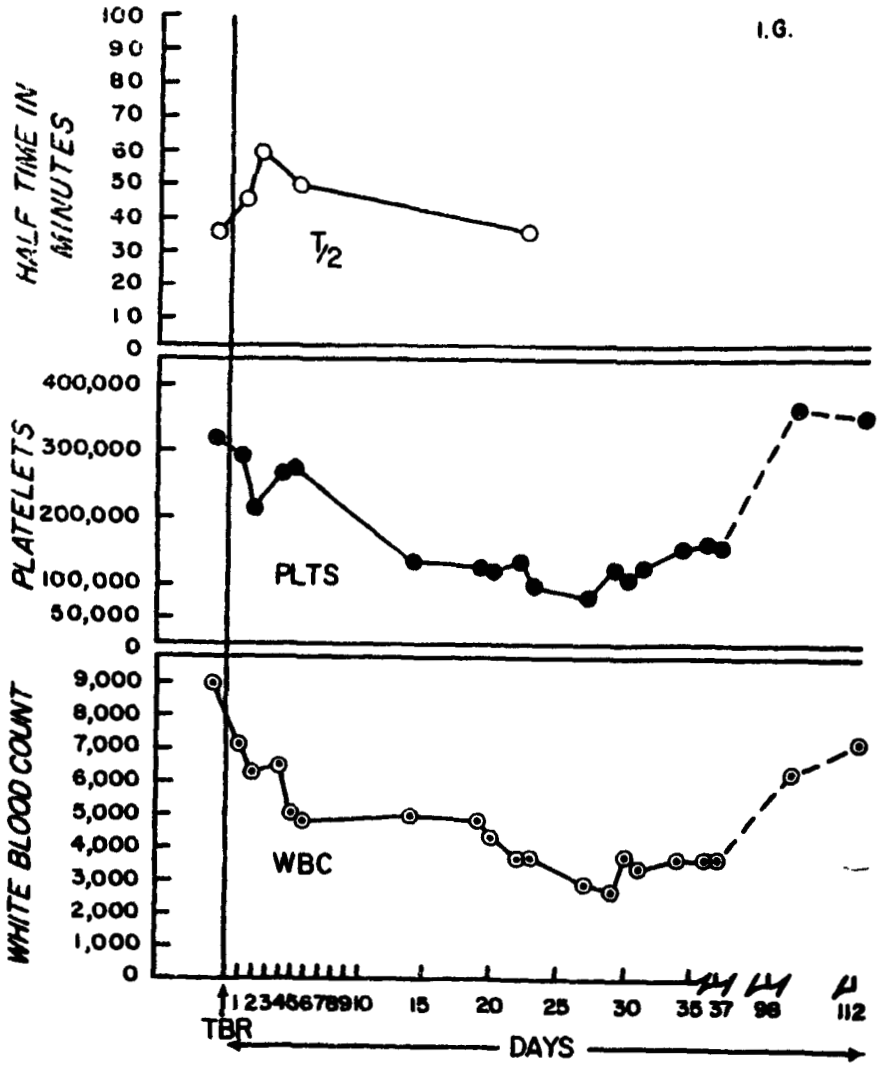

I.G.
HALF TIME IN MINUTES
100
90
80
70
60
50
40
30
20
10
0
T/2
PLATELETS
400,000
300,000
200,000
100,000
50,000
0
PLTS
WHITE BLOOD COUNT
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
WBC
1 2 3 4 5 6 7 8 9 10 15 20 25 30 35 37 98 112
TBR
DAYS

Figure V

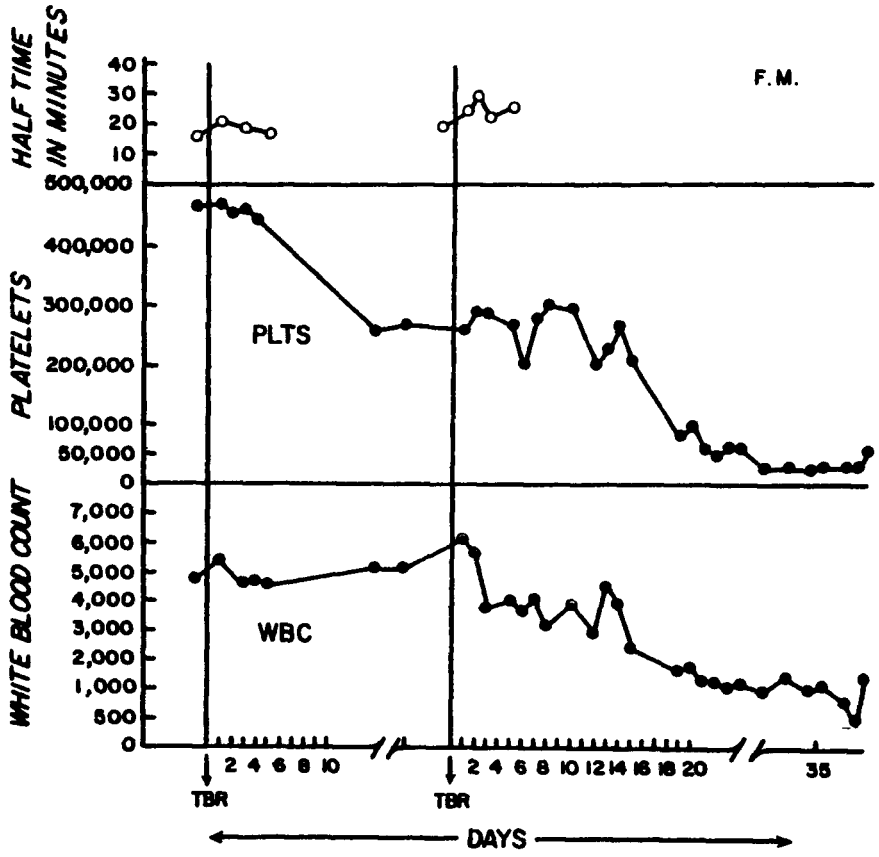
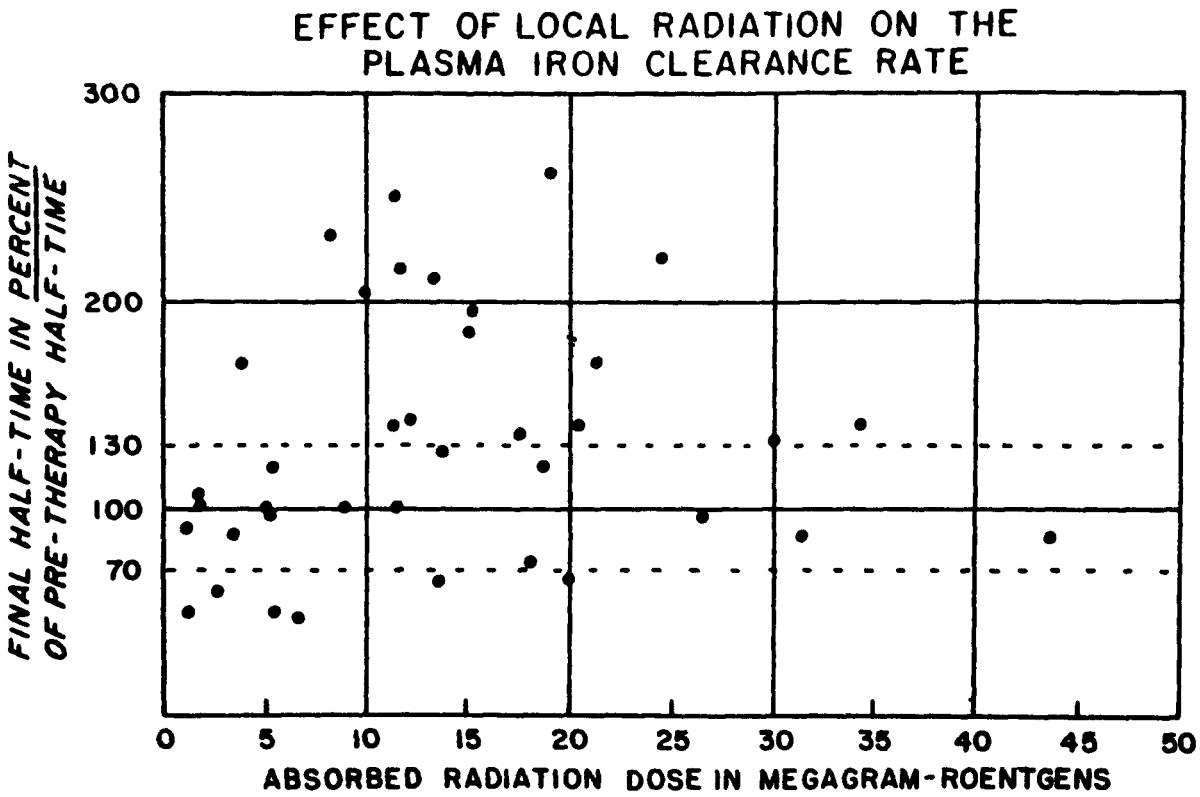

HALF TIME IN MINUTES
40
30
20
10
F.M.
PLATELETS
500,000
400,000
300,000
200,000
100,000
50,000
0
PLTS
WHITE BLOOD COUNT
7,000
6,000
5,000
4,000
3,000
2,000
1,000
500
0
WBC
2 4 6 8 10
2 4 6 8 10 12 14 16 18 20
35
TBR
TBR
DAYS

Figure VI

DOSE	SYMPTOMS	WBC	PLATELETS	FE
100 R	0/1	1/1	1/1	1/1
125 R	0/1	1/1		1/1
150 R	0/1	1/1	0/1	1/1
200 R INCLUDING 1ST RX.	7/23	16/23	16/19	11/17
200 R + 100 R (5 WKS)	0/1	0/1	0/1	0/1
200 R + 200 R (5 MOS)	1/1	1/1	1/1	1/1
200 R + 200 R (3 WKS)	1/1	1/1	1/1	
50 R x 5	0/3	3/3	3/3	1/2
55 R x 10	1/1	1/1	1/1	1/1

x/y indicates number of patients showing a change out of the number studied for the item in question.

Table II


EFFECT OF LOCAL RADIATION ON THE
PLASMA IRON CLEARANCE RATE
FINAL HALF-TIME IN PERCENT
OF PRE-THERAPY HALF-TIME
300
200
130
100
70
0
5
10
15
20
25
30
35
40
45
50
ABSORBED RADIATION DOSE IN MEGAGRAM-ROENTGENS

PATIENT J.M.
TUMOR DOSE - 1500 R/3 WKS
INTEGRAL DOSE - 26.6 MGR/3 WKS

	PRE-THERAPY	END 1ST WEEK	END 2ND WEEK	END 3RD WEEK
WBC	14,500	9,300	5,500	5,250
PLATELETS	236,000	224,000	194,000	166,000
HGB.	12.8	13.3	12.0	11.9
T/2	82'	75'	127'	76'
FE_C	85	115	127	93

PATIENT W.M.
TUMOR DOSE - 1500 R/3 WKS
INTEGRAL DOSE - 31.4 MGR/3 WKS

	PRE-THERAPY	END 1ST WEEK	END 2ND WEEK	END 3RD WEEK
WBC	12,700	6,900	7,400	4,950
PLATELETS	264,000	288,000	184,000	256,000
HGB.	13.8	14.2	13.5	12.6
T/2	102'	142'	145'	87'
FE_C	121	149	164	103

Table III

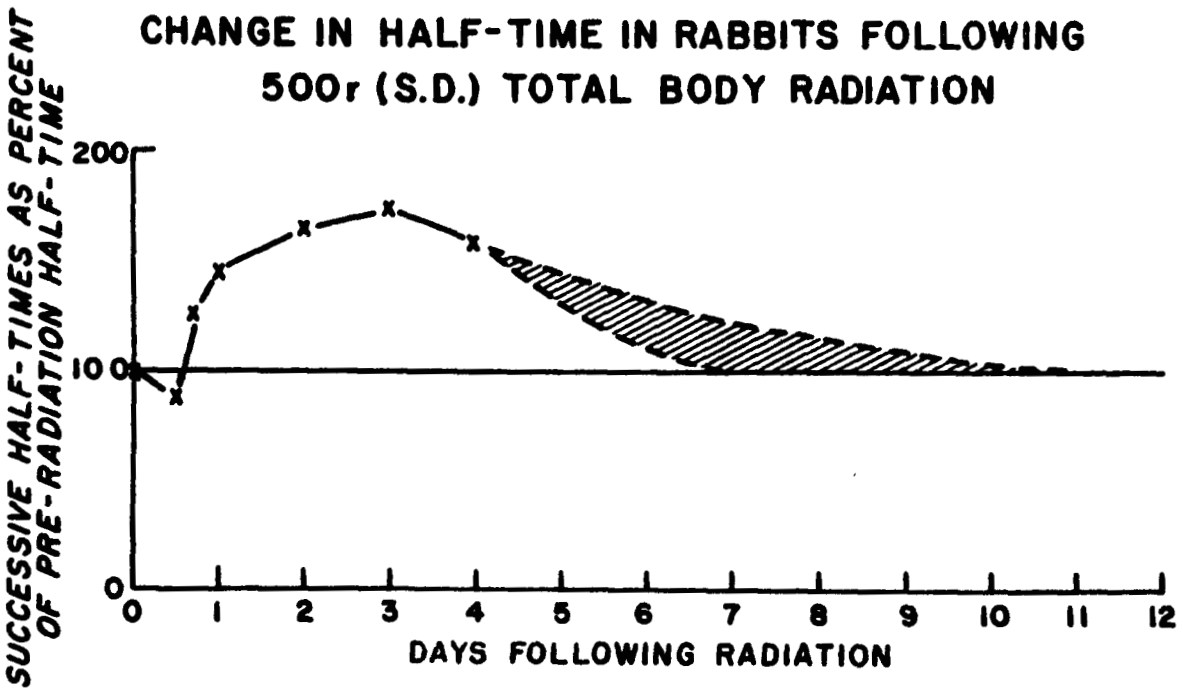

CHANGE IN HALF-TIME IN RABBITS FOLLOWING
500r (S.D.) TOTAL BODY RADIATION
SUCCESSIVE HALF-TIMES AS PERCENT OF PRE-RADIATION HALF-TIME
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS FOLLOWING RADIATION

Figure VIII

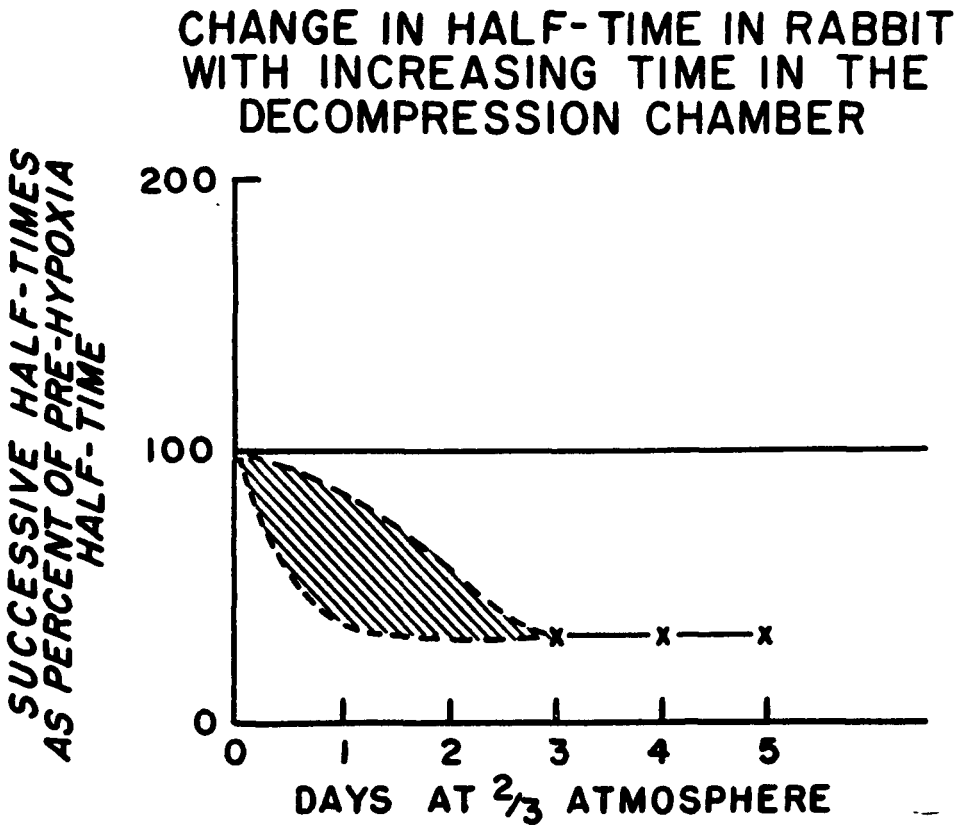

CHANGE IN HALF-TIME IN RABBITS
WITH INCREASING TIME IN THE
DECOMPRESSION CHAMBER
SUCCESSIVE HALF-TIMES
AS PERCENT OF PRE-HYPOXIA
HALF-TIME
200
100
0
0
1
2
3
4
5
DAYS AT 2/3 ATMOSPHERE

Figure IX

CHANGE IN HALF-TIME IN RABBITS HAVING RECEIVED 500 r (S.D.) TOTAL BODY RADIATION, WITH THREE DAYS OF HYPOXIA PRIOR TO SECOND HALF-TIME DETERMINATION

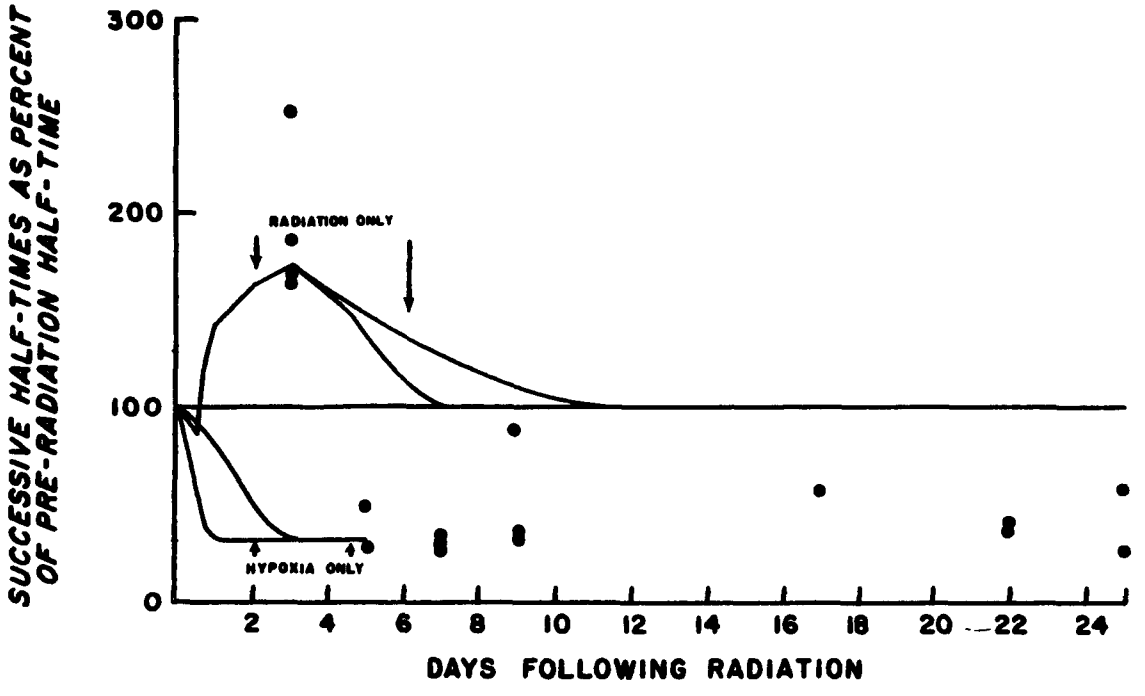


Figure X

APPENDIX #4

Investigations of Enzymic X-ray Dosimeters in Red Blood Cells

From the Department of Radiology, Baylor University College of Medicine, Houston, Texas.

INVESTIGATIONS OF ENZYMIC X-RAY DOSIMETERS IN RED BLOOD CELLS

D. A. RAPPOPORT and V. P. COLLINS

I. Glutathione Biosynthetic System

The present report is a summary of the preliminary findings on the formation of glutathione by particulates from hemolysates of rabbit, dog and human subjects. These results are essentially a confirmation of the work of Dimant, Landsberg and London (J. Biol. Chem., 213, 769 (1955)) who first found this system in erythrocytes.

Experimental

Blood was obtained by cardiac puncture from rabbits, by arterial puncture from dogs and by venipuncture from normal human subjects. The erythrocytes were separated from plasma by centrifugation, washed four times with cold isotonic saline and then lysed with an equal volume of deionized water. The hemolysate was then centrifuged at 25,000 x g for 20 minutes in a refrigerated Servall centrifuge and the gel-like pellet was separated from the supernatant and resuspended in an equal volume of isotonic saline.

Incubation mixtures were prepared using 2.5 ml. of resuspended red cell particulate and 2.5 ml. of buffer and additives. Both "tris"-buffer and phosphate buffer (both 0.05M at pH 7.4) were used. The additives consisted of the following components:

L-glutamic acid	0.008M
L-cysteine	0.008M
$MgSO_4$	0.01M
KCl	0.02M

glucose, glycogen or phosphoglyceric acid	0.03M
2-C^{14}-glycine	0.004M
ATP	0.0016M

The incubations were carried out in a Dubnoff metabolic shaker for 3 hours at 37° C.

After incubation, the proteins were precipitated with cold trichloracetic acid and the supernatant was desalted on Dowex 2 resin (oH-form). The desalted solutions were then treated with one or two drops of 30 percent hydrogen peroxide to oxidize reduced glutathione. The solution was then concentrated to a small volume (approximately 0.2 ml.) on a steam bath and subsequently aliquots were placed on Whatman 3MM paper for descending chromatography. t-Butanol, formic acid and water (75:15:15) was the developing solvent which separated 2-C^{14}-glycine (Rf 0.35) from oxidized C^{14}-glutathione (Rf 0.08). The glutathione was then eluted from the paper, concentrated on the steam bath to a small volume and then transferred to stainless steel planchets for radioactive determination with an ultra thin-window gas flow GM tube (Nuclear).

Results and Discussion

With 2-C^{14}-glycine, hemolysates of rabbit, dog and human subjects synthesized glutathione in vitro. The best results were obtained in incubates with phosphate buffer and glucose as an energy source. This data and findings are listed below.

Expt. No.	Source of Blood	Energy Substrate and Buffer	μM oxid. Glutathione per ml. incubate
1	Dog	glycogen,ph-buffer	0.46
2	Dog	glycogen,ph-buffer	0.45
3	Rabbit	glucose,tris-buffer	0.32

(contd.)

Expt. No.	Source of Blood	Energy Substrate and Buffer	µM oxid. Glutathione per ml. incubate
4	Rabbit	glucose, tris-buffer	0.26
5	Rabbit	glucose, ph-buffer	0.49
6	Rabbit	ph-glycerate, tris-buffer	0.16
7	Rabbit	ph-glycerate, tris-buffer	0.16
8	Human	glucose, ph-buffer	0.34

Certain advantages and disadvantages are inherent in the glutathione biosynthetic system. The advantage is that a sulfhydryl compound is formed, hence it is more likely to be influenced by X-radiation. The disadvantage in this system is the laborious technique in isolating and analyzing the product. However, it is the primary purpose of these studies to find a radiation sensitive enzymic system first, then it is planned to investigate better methods of analysis.

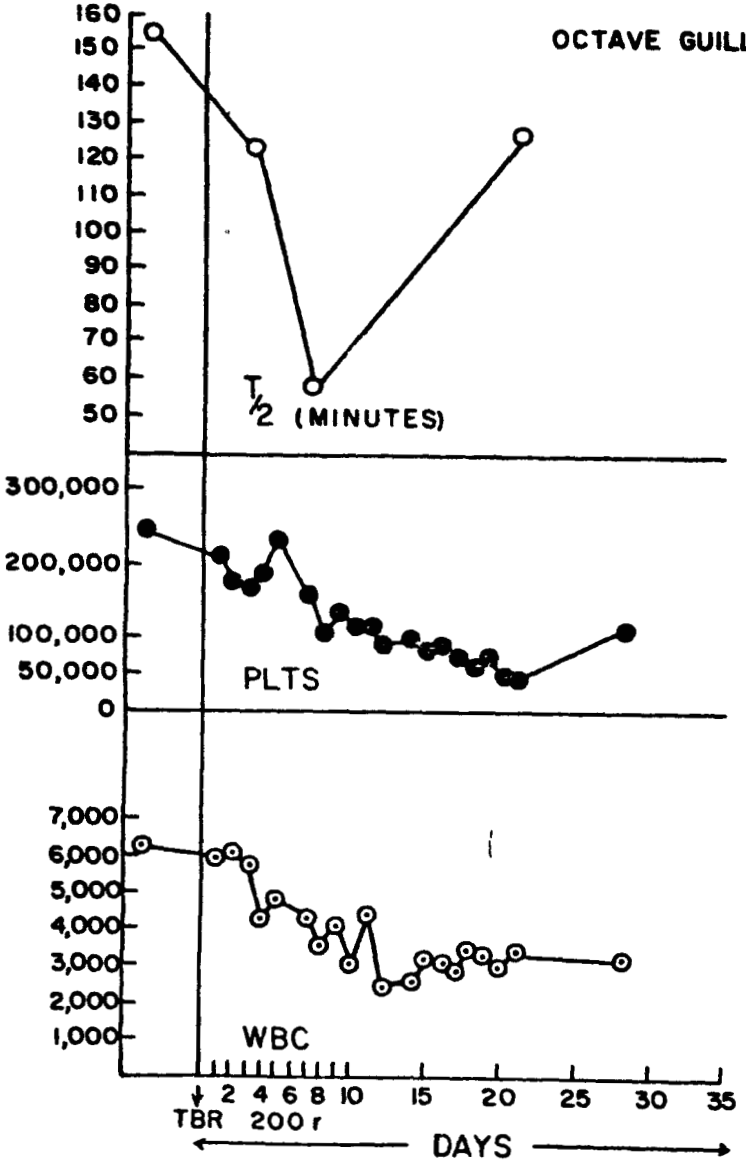
Since the preliminary study was essentially a carrier-free analysis of glutathione, at this time an isotope dilution technique is under investigation. Also, irradiation studies are now initiated to study the influence of varying doses of total body radiation (rabbits) on the biosynthesis of glutathione by red cell stroma.

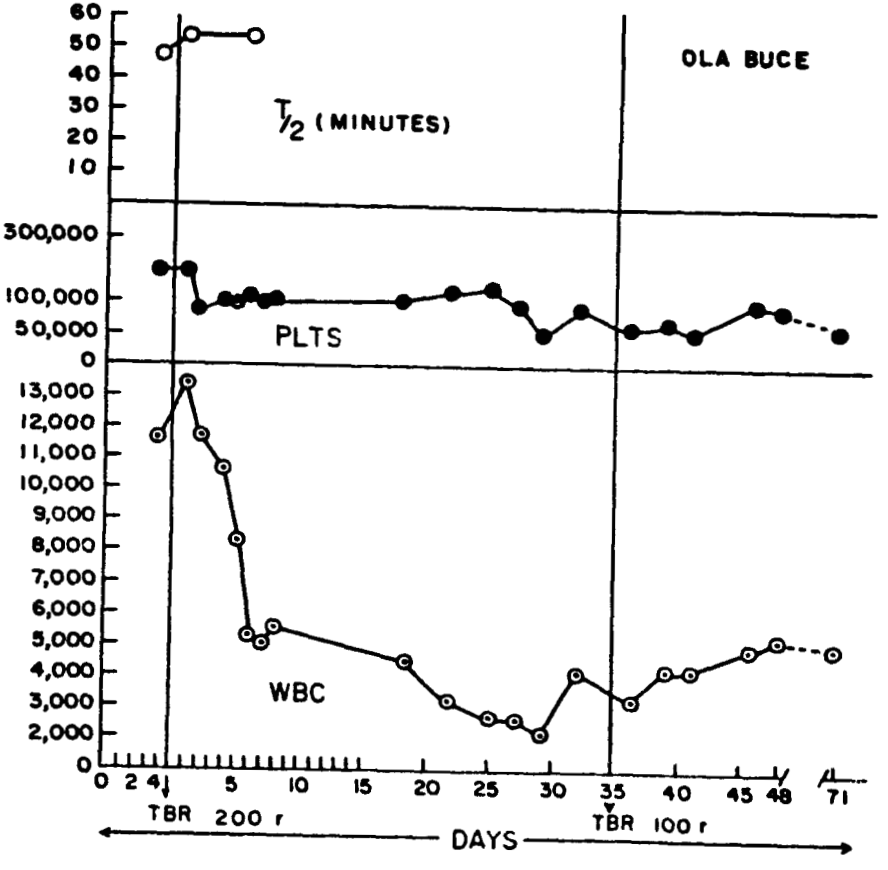
In addition to the above glutathione system, a study of radiation effects on nucleotide (ATP and DPN) hydrolyzing enzymes in red cell stroma has also been initiated.

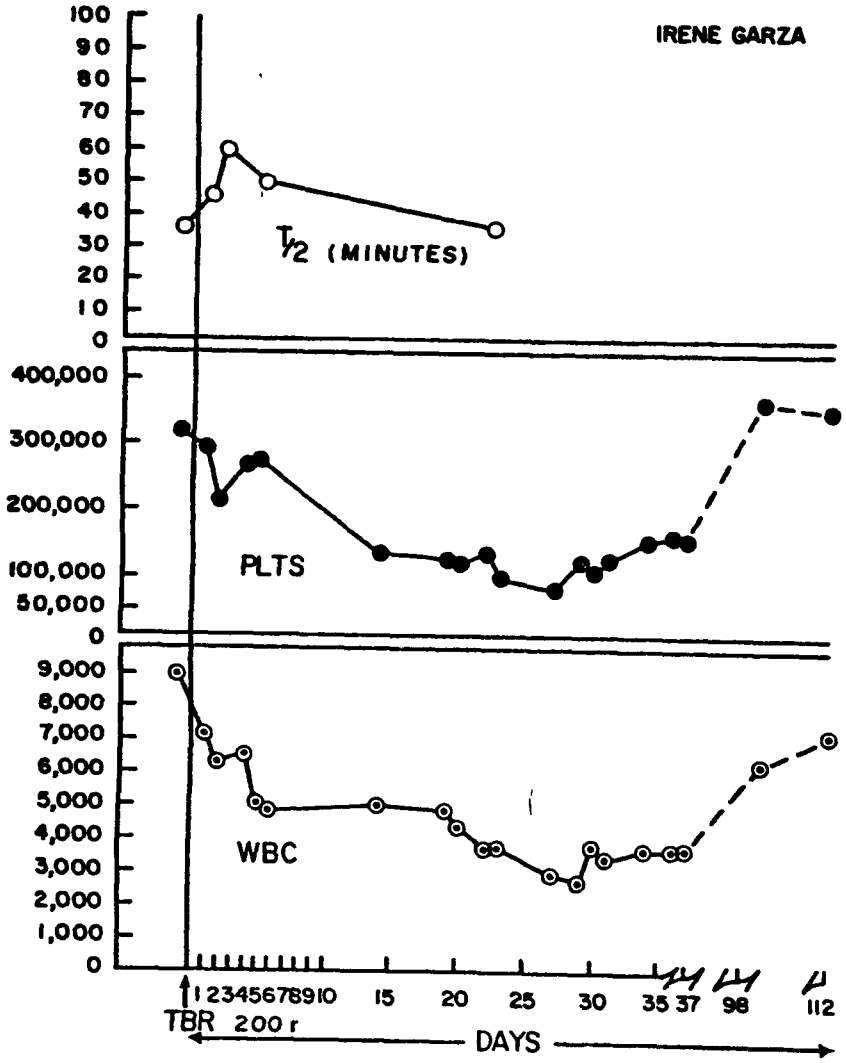
APPENDIX #5

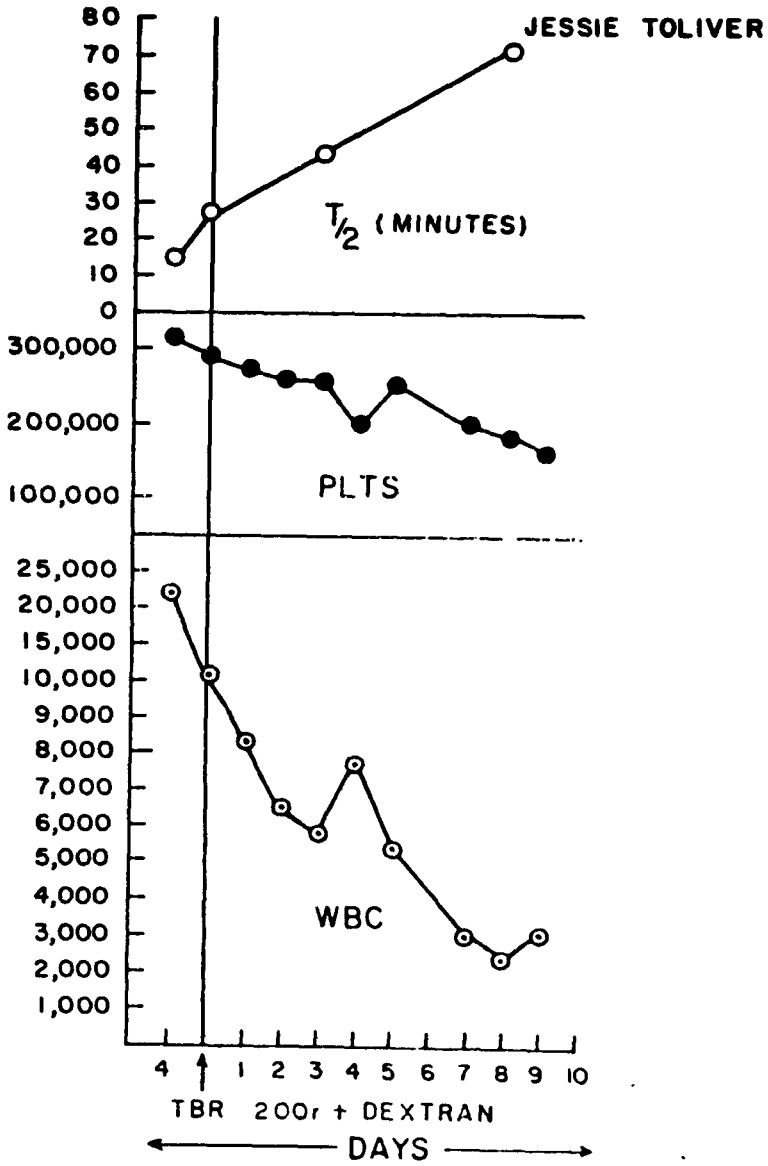
Effects of Total Body Radiation on Iron Turnover, Platelet Count and White Cell Count

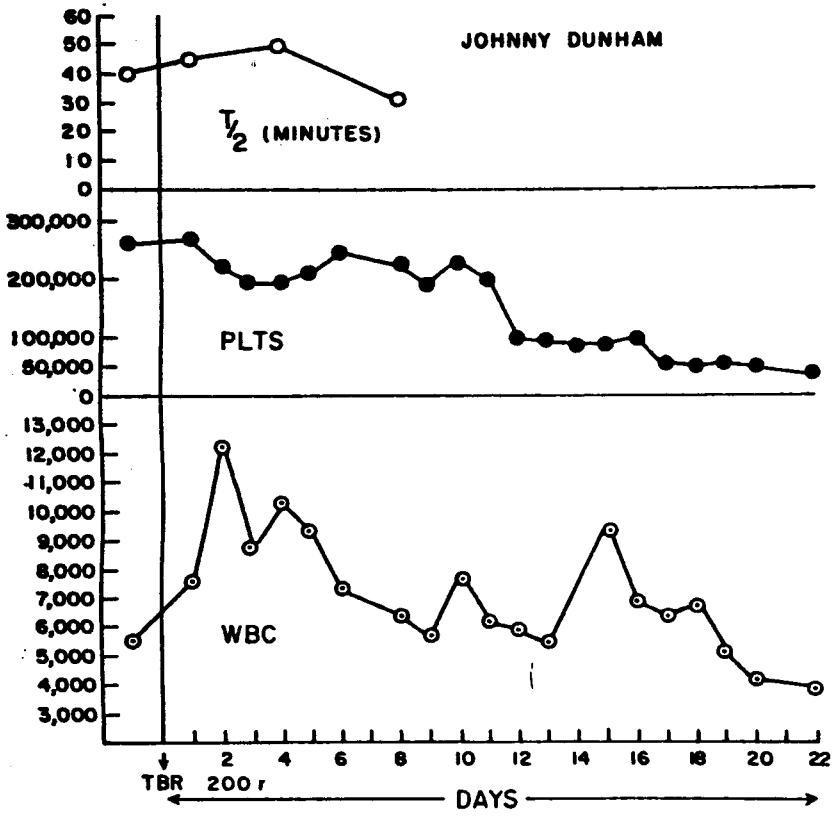
From the Department of Radiology, Baylor University College of Medicine, Houston, Texas.

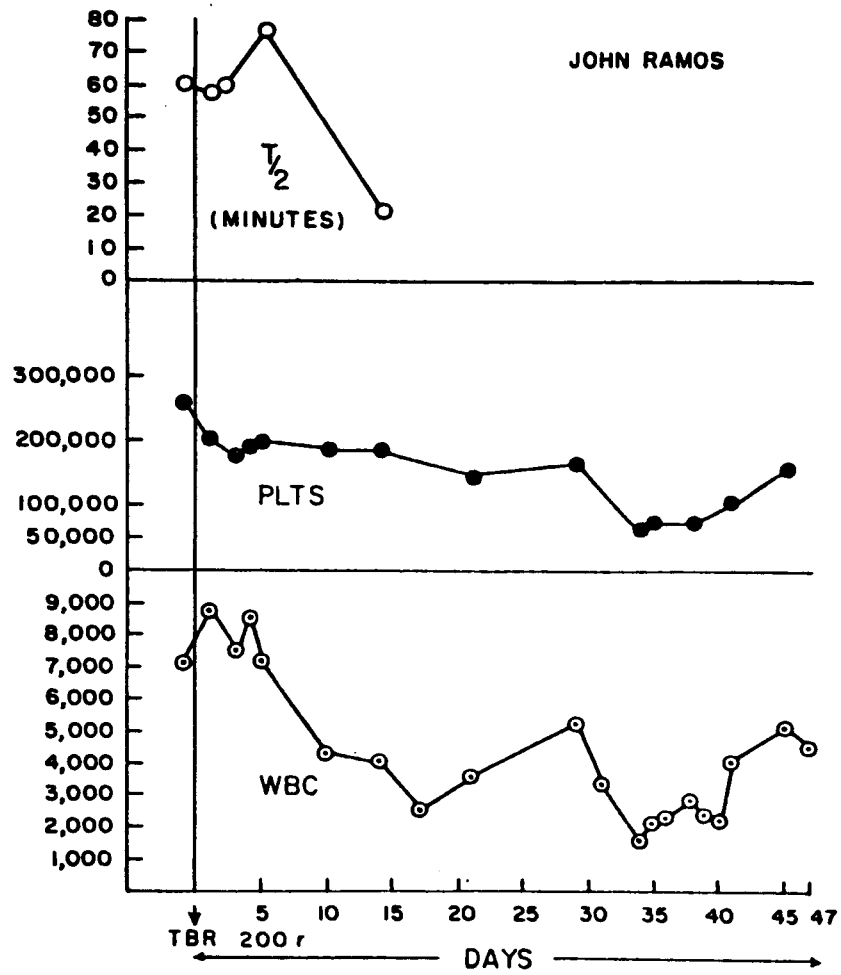

OCTAVE GUILLORY
160
150
140
130
120
110
100
90
80
70
60
50
T/2 (MINUTES)
300,000
200,000
100,000
50,000
0
PLTS
7,000
6,000
5,000
4,000
3,000
2,000
1,000
WBC
2 4 6 8 10 15 20 25 30 35
TBR 200 r
DAYS

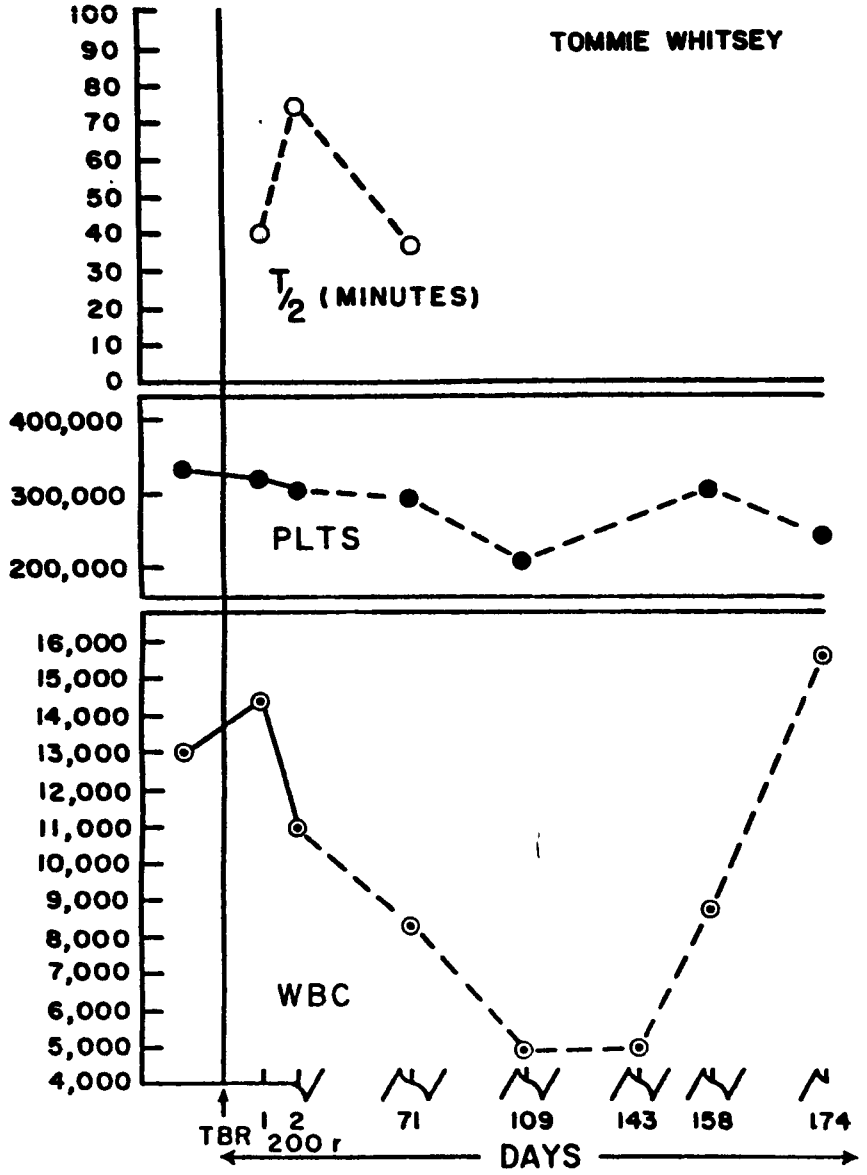

OLA BUCE
60
50
40
30
20
10
T/2 (MINUTES)
300,000
100,000
50,000
0
PLTS
13,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
0
WBC
0 2 4 5 10 15 20 25 30 35 40 45 48 71
TBR 200 r
TBR 100 r
DAYS

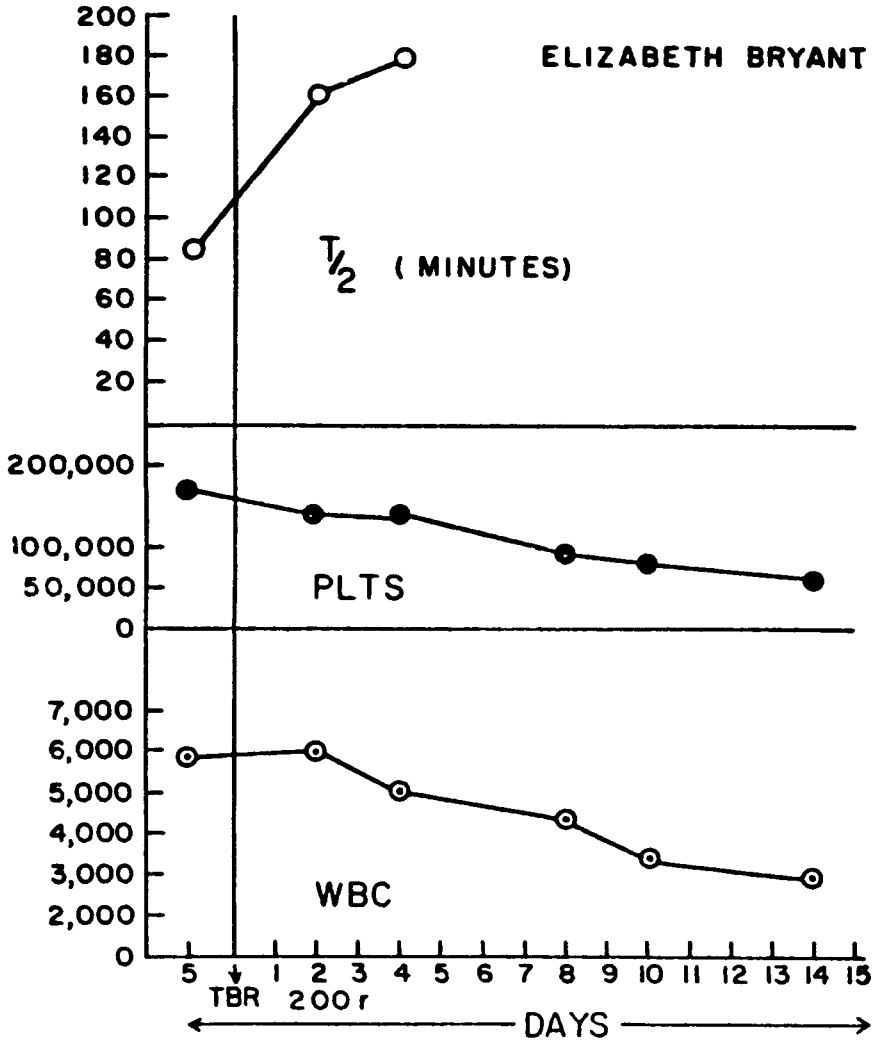

IRENE GARZA
100
90
80
70
60
50
40
30
20
10
0
T/2 (MINUTES)
400,000
300,000
200,000
100,000
50,000
0
PLTS
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
WBC
1 2 3 4 5 6 7 8 9 10 15 20 25 30 35 37 98 112
TBR 200 r
DAYS

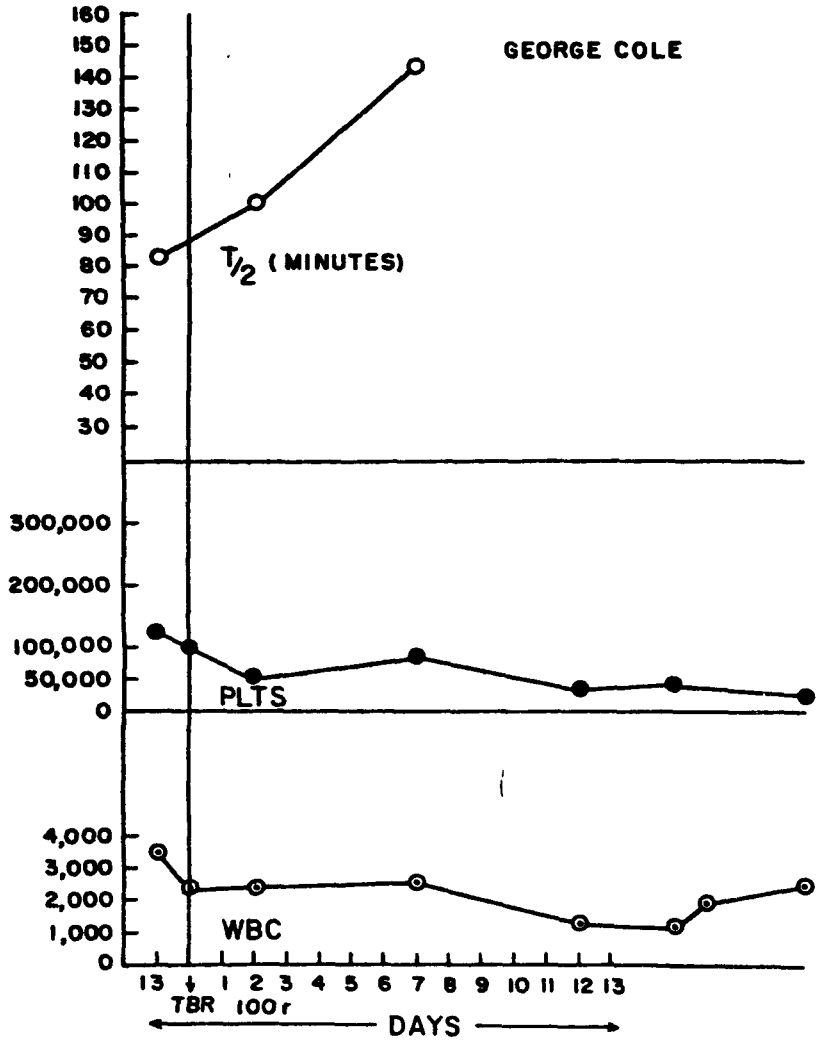

JESSIE TOLIVER
80
70
60
50
40
30
20
10
0
T/2 (MINUTES)
300,000
200,000
100,000
PLTS
25,000
20,000
15,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
WBC
4 1 2 3 4 5 6 7 8 9 10
TBR 200r + DEXTRAN
DAYS

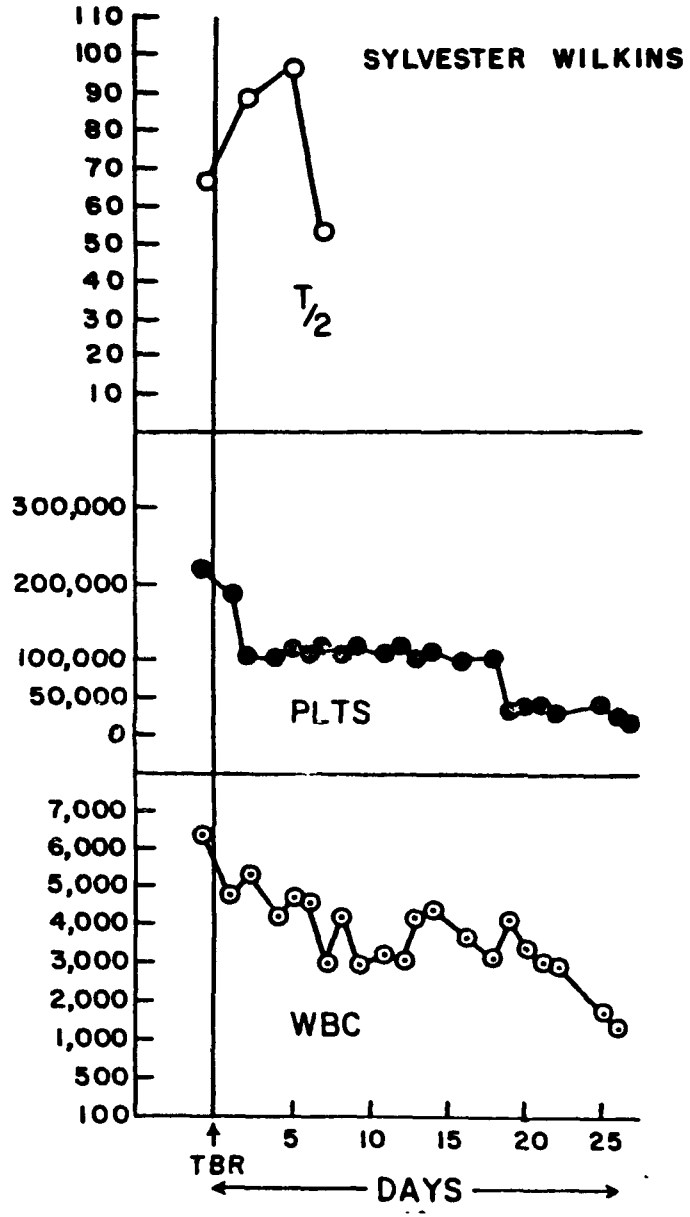

JOHNNY DUNHAM
T½ (MINUTES)
PLTS
WBC
TBR 200 r
DAYS

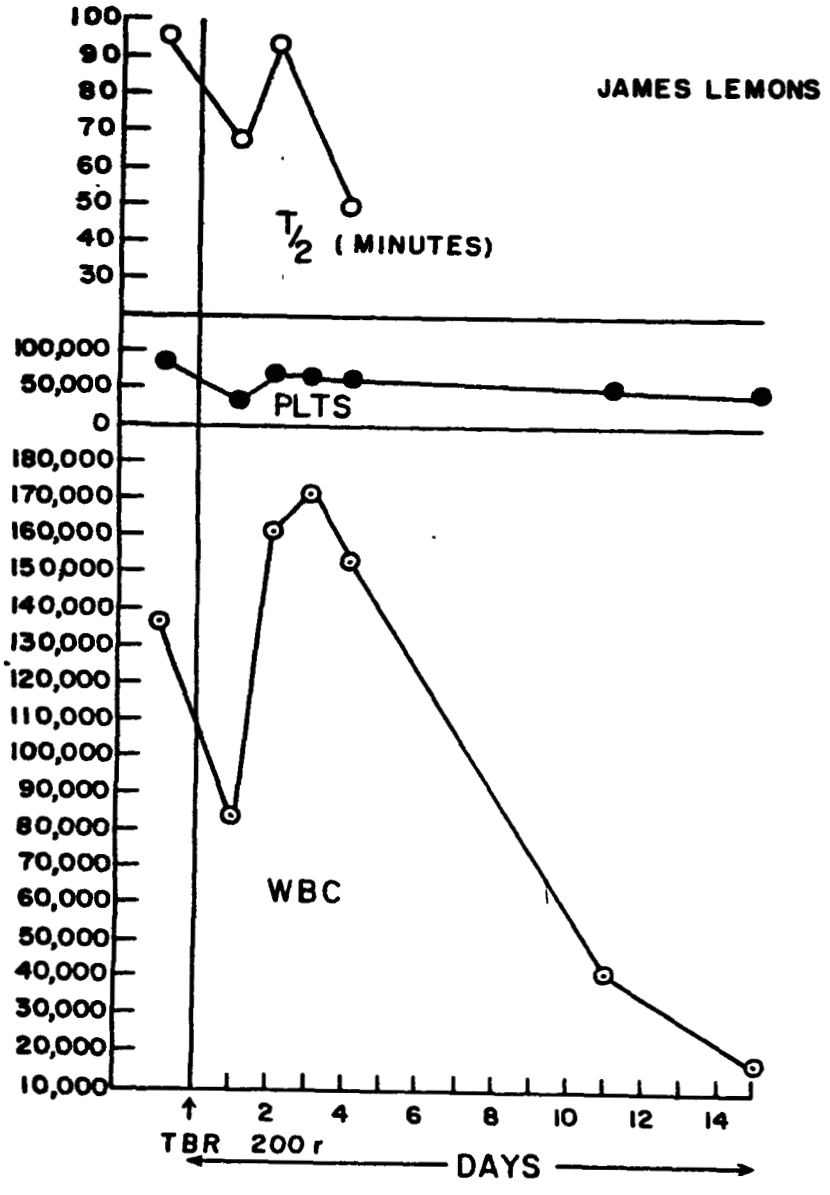

JOHN RAMOS
T½
(MINUTES)
PLTS
WBC
TBR 200 r
DAYS

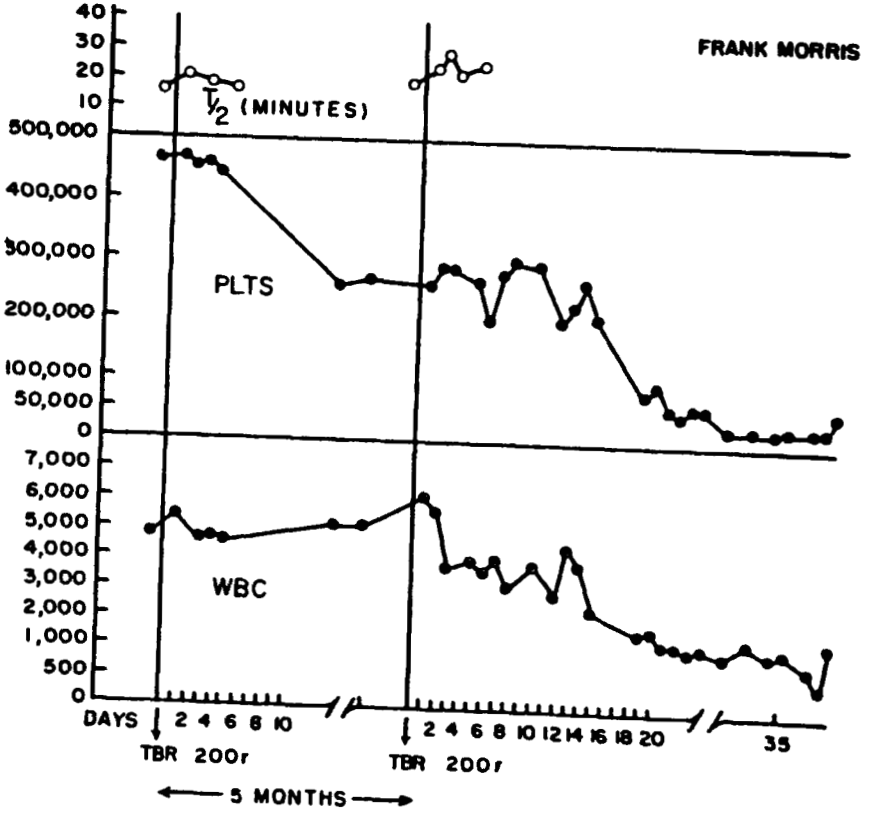

TOMMIE WHITSEY
100
90
80
70
60
50
40
30
20
10
0
T/2 (MINUTES)
400,000
300,000
200,000
PLTS
16,000
15,000
14,000
13,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
WBC
TBR 1 2 71 109 143 158 174
200 r
DAYS

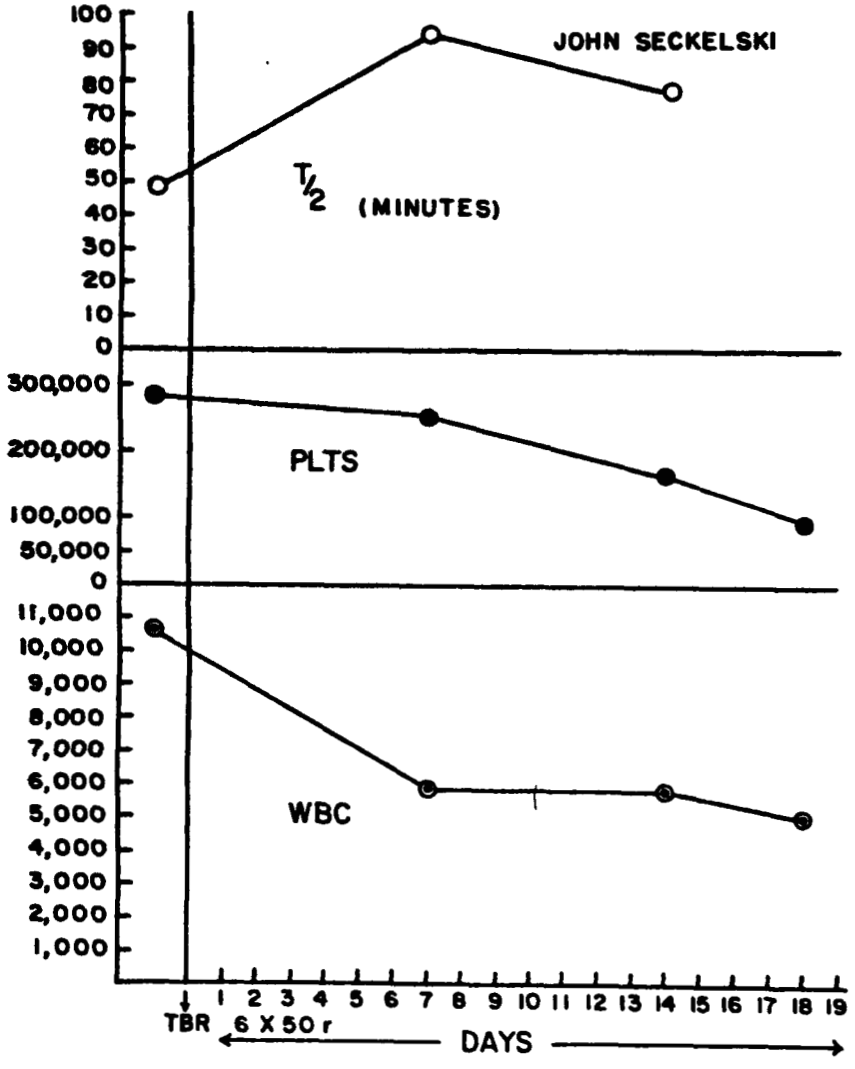

ELIZABETH BRYANT
200
180
160
140
120
100
80
60
40
20
T/2 (MINUTES)
200,000
100,000
50,000
0
PLTS
7,000
6,000
5,000
4,000
3,000
2,000
0
WBC
5 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TBR
200 r
DAYS

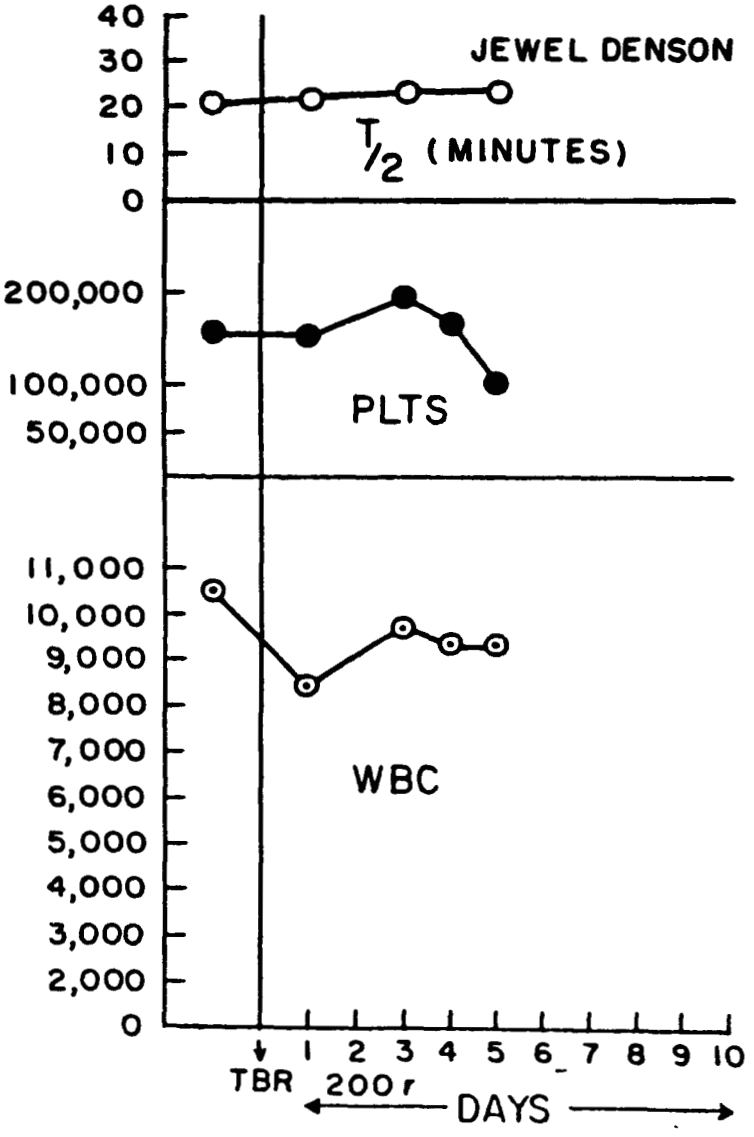

GEORGE COLE
160
150
140
130
120
110
100
90
80
70
60
50
40
30
T/2 (MINUTES)
300,000
200,000
100,000
50,000
0
PLTS
4,000
3,000
2,000
1,000
0
WBC
13 1 2 3 4 5 6 7 8 9 10 11 12 13
TBR 100r
DAYS

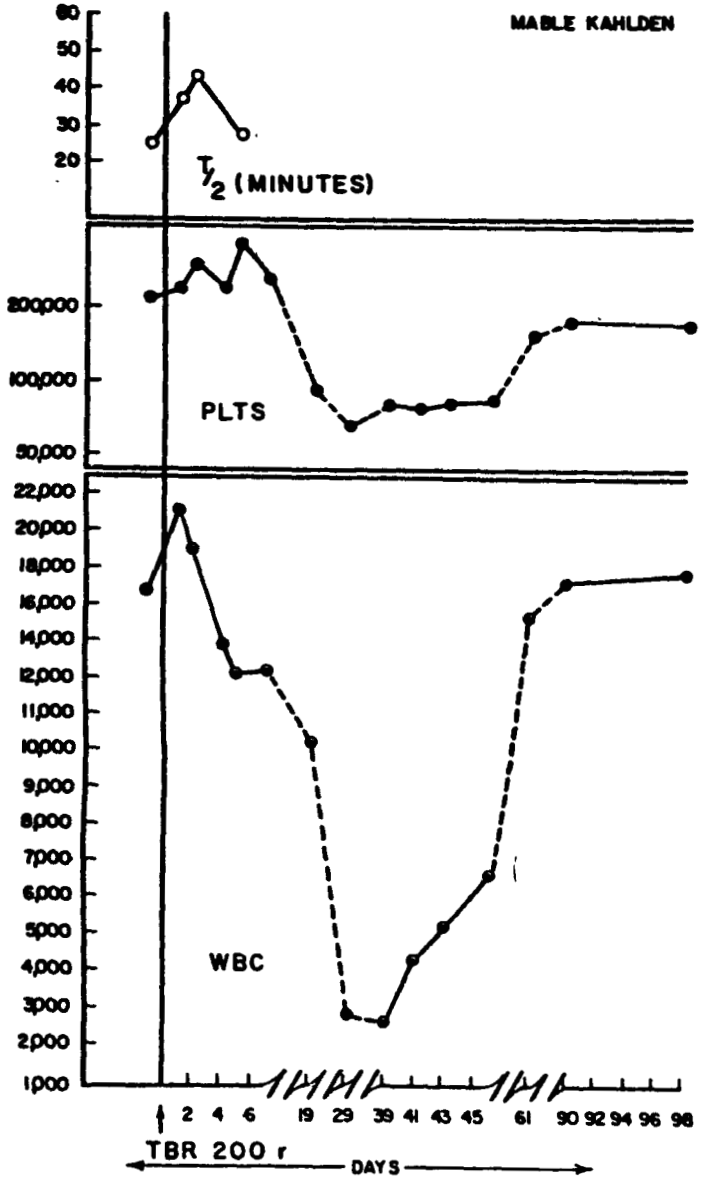

SYLVESTER WILKINS
110
100
90
80
70
60
50
40
30
20
10
T/2
300,000
200,000
100,000
50,000
0
PLTS
7,000
6,000
5,000
4,000
3,000
2,000
1,000
500
100
WBC
5
10
15
20
25
TBR
DAYS

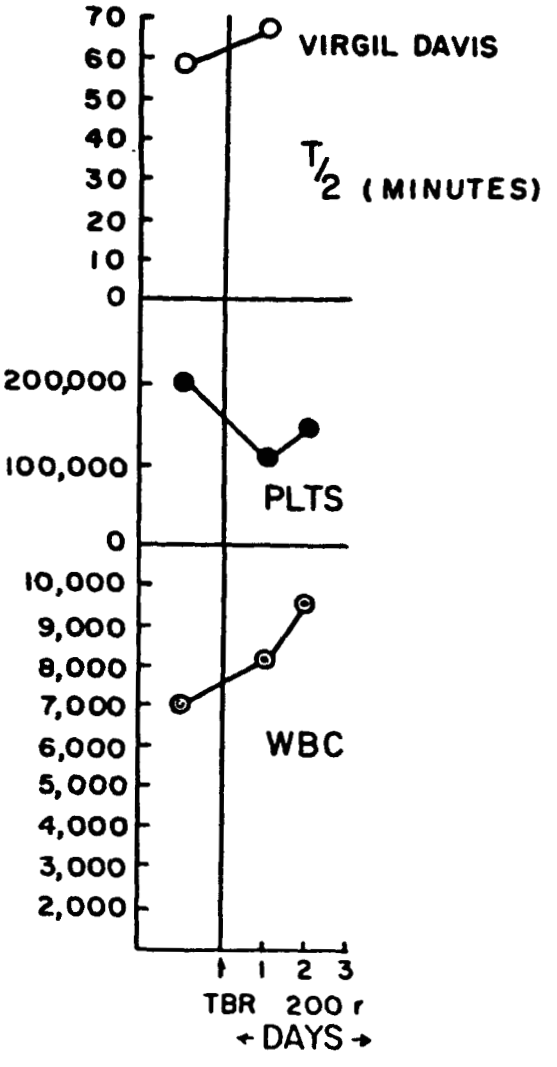

JAMES LEMONS
100
90
80
70
60
50
40
30
T/2 (MINUTES)
100,000
50,000
0
PLTS
180,000
170,000
160,000
150,000
140,000
130,000
120,000
110,000
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
WBC
2
4
6
8
10
12
14
TBR 200r
DAYS

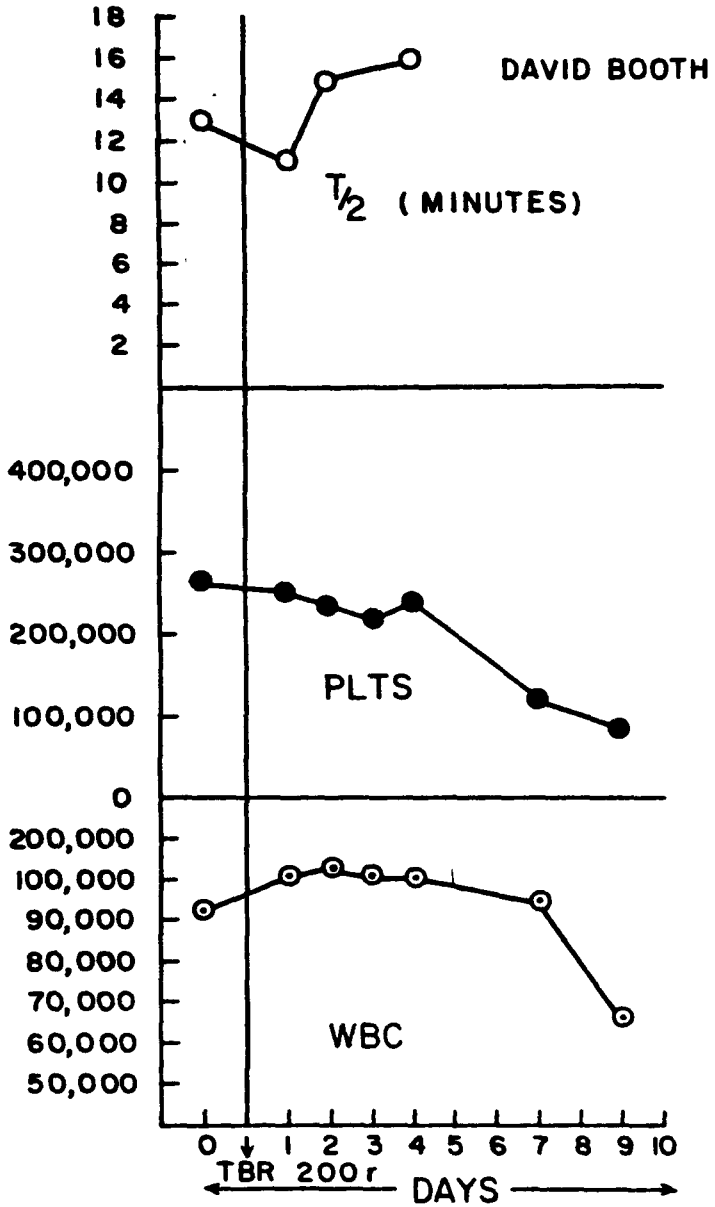

FRANK MORRIS
40
30
20
10
T/2 (MINUTES)
500,000
400,000
300,000
200,000
100,000
50,000
0
PLTS
7,000
6,000
5,000
4,000
3,000
2,000
1,000
500
0
WBC
DAYS
2 4 6 8 10
2 4 6 8 10 12 14 16 18 20
35
TBR 200r
TBR 200r
5 MONTHS

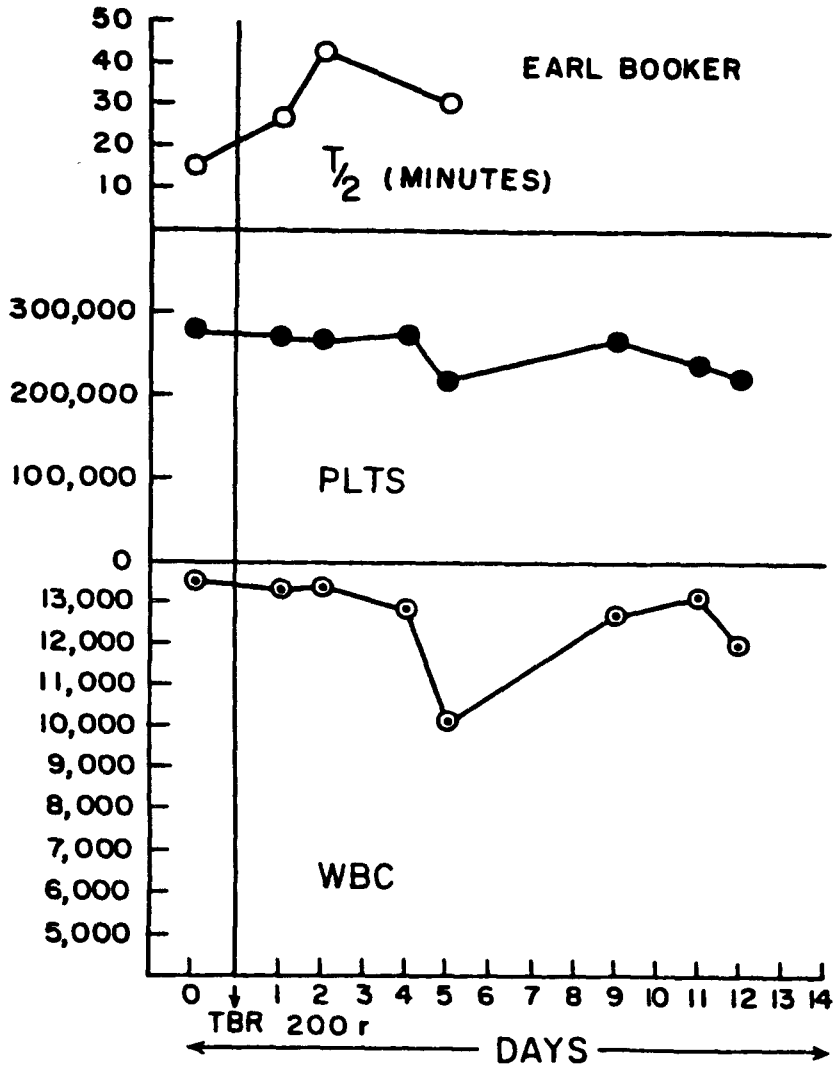

JOHN SECKELSKI
T½ (MINUTES)
PLTS
WBC
100
90
80
70
60
50
40
30
20
10
0
300,000
200,000
100,000
50,000
0
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TBR
6 X 50 r
DAYS

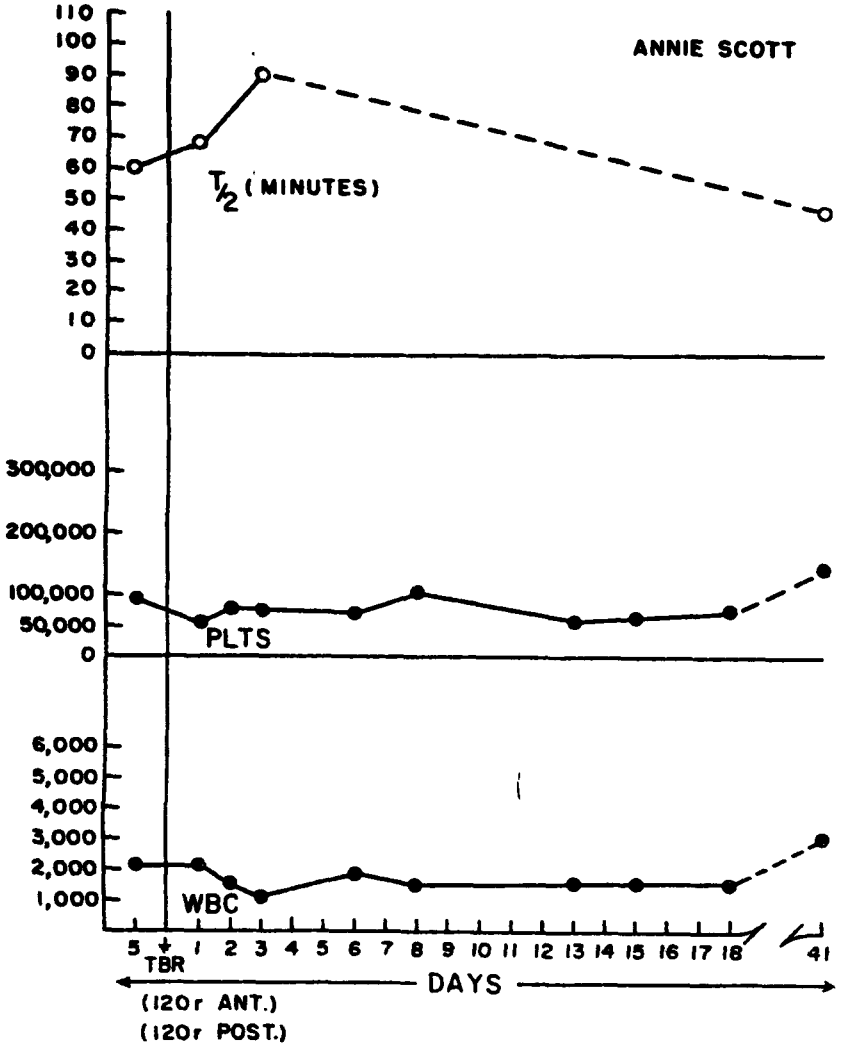

JEWEL DENSON
T½ (MINUTES)
PLTS
WBC
40
30
20
10
0
200,000
100,000
50,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
0
1 2 3 4 5 6 7 8 9 10
TBR
200 r
DAYS

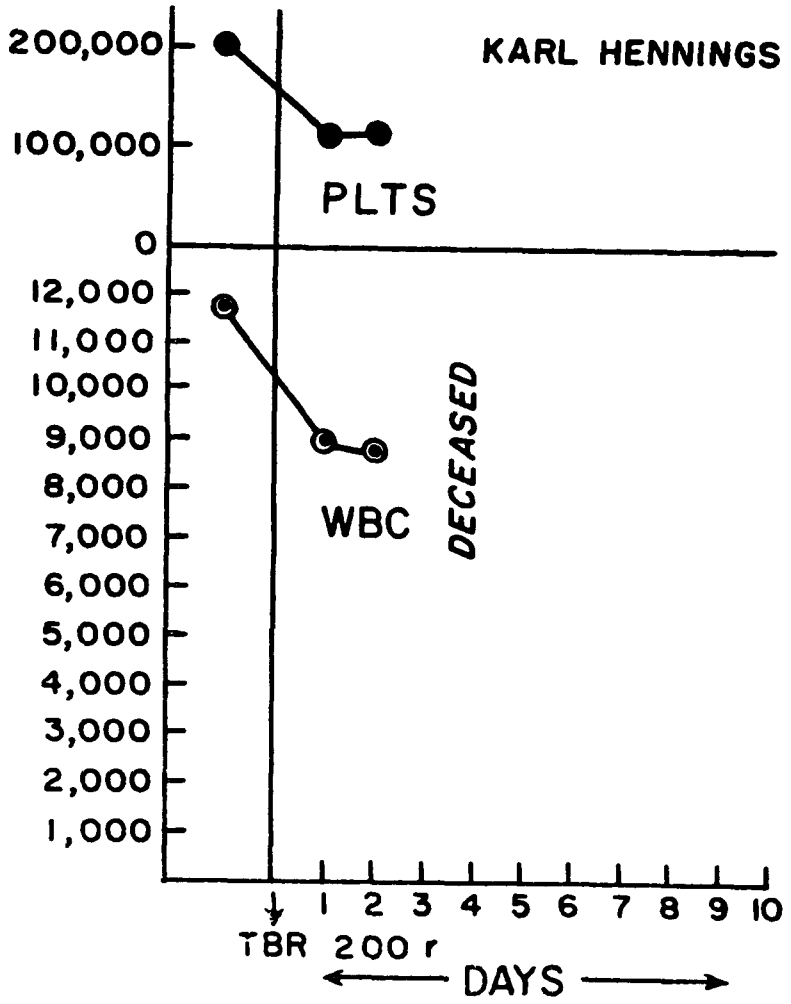

MABLE KAHLDEN
80
50
40
30
20
T/2 (MINUTES)
200,000
100,000
50,000
PLTS
22,000
20,000
18,000
16,000
14,000
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
WBC
2 4 6 19 29 39 41 43 45 61 90 92 94 96 98
TBR 200 r
DAYS

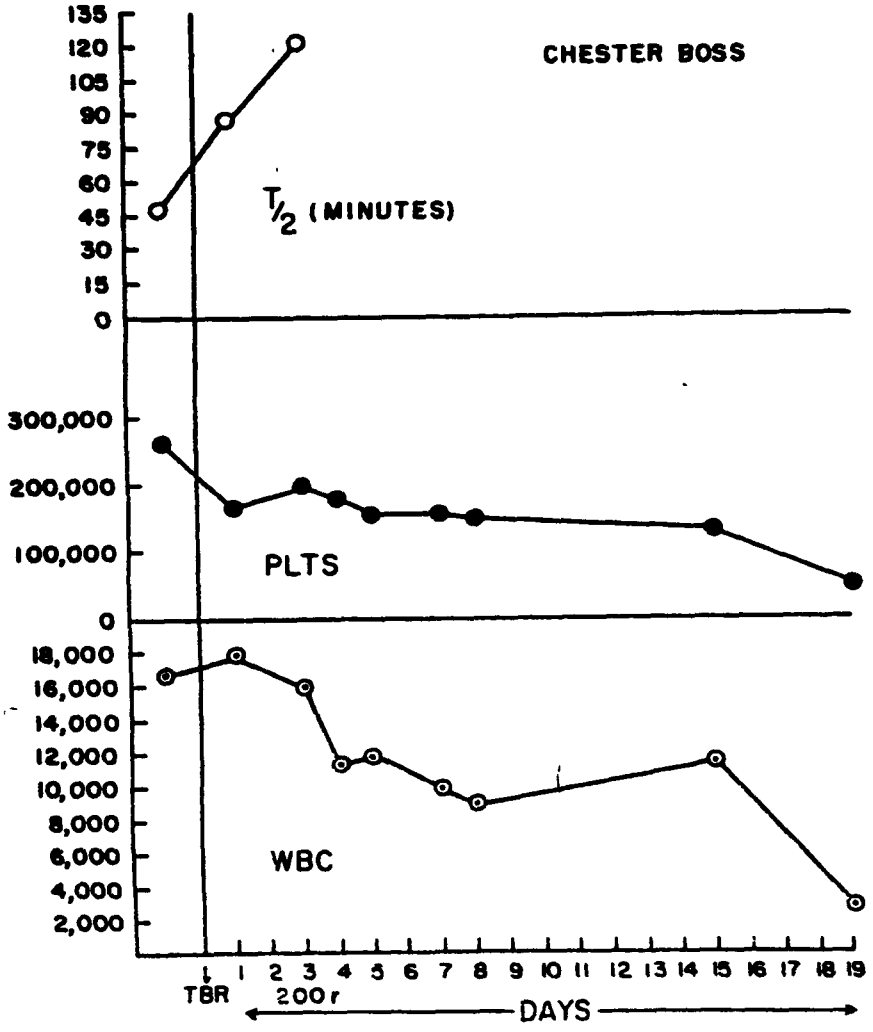

VIRGIL DAVIS
70
60
50
40
30
20
10
0
T/2 (MINUTES)
200,000
100,000
0
PLTS
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
WBC
1 2 3
TBR 200 r
DAYS

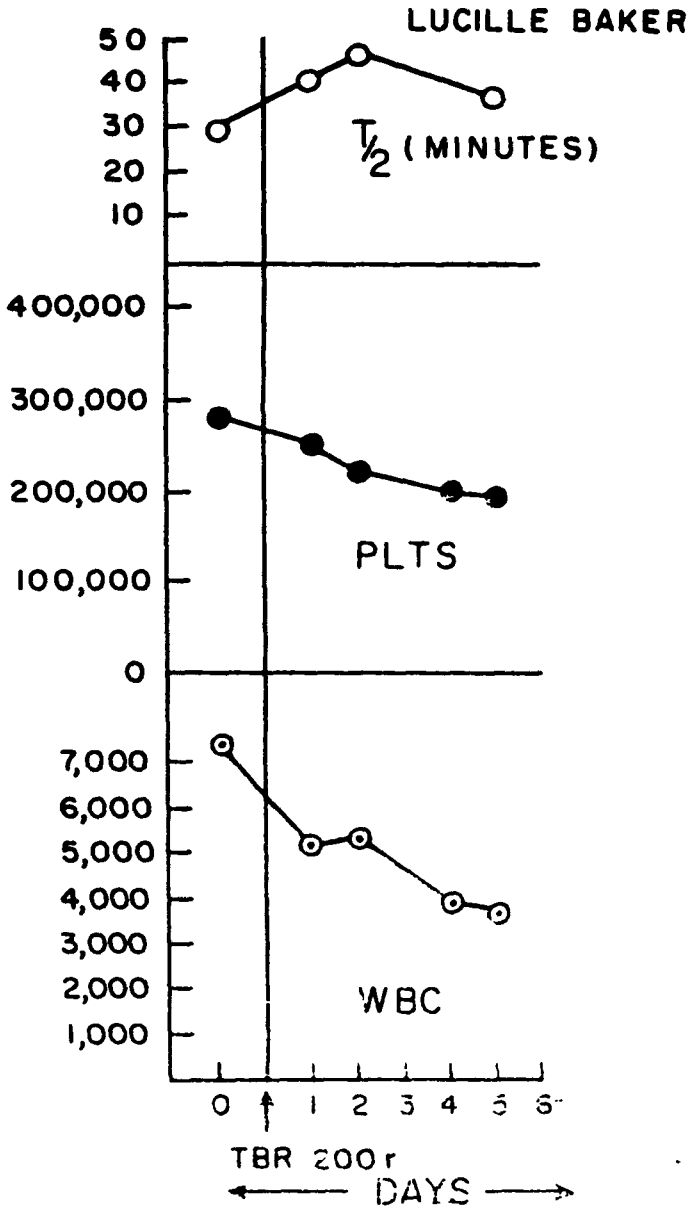

DAVID BOOTH
T/2 (MINUTES)
PLTS
WBC
TBR 200r
DAYS


EARL BOOKER
T/2 (MINUTES)
PLTS
WBC
TBR 200r
DAYS


ANNIE SCOTT
110
100
90
80
70
60
50
40
30
20
10
0
$T_{1/2}$ (MINUTES)
300,000
200,000
100,000
50,000
0
PLTS
6,000
5,000
4,000
3,000
2,000
1,000
WBC
5
TBR
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
41
DAYS
(120r ANT.)
(120r POST.)


KARL HENNINGS
200,000
100,000
0
PLTS
12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
WBC
DECEASED
1 2 3 4 5 6 7 8 9 10
TBR 200r
DAYS


CHESTER BOSS
135
120
105
90
75
60
45
30
15
0
T/2 (MINUTES)
300,000
200,000
100,000
0
PLTS
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
WBC
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
TBR
200r
DAYS


LUCILLE BAKER
50
40
30
20
10
T/2 (MINUTES)
400,000
300,000
200,000
100,000
0
PLTS
7,000
6,000
5,000
4,000
3,000
2,000
1,000
WBC
0 1 2 3 4 5 6
TBR 200r
DAYS